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NEW PRINCIPLES IN THERAPEUTIC INOCULATION

*Being an Expansion of a Lecture delivered at a Special Meeting
of the Royal Society of Medicine on Nov. 30th, 1922*

BY

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In Collaboration with

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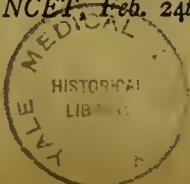
AND

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NEW PRINCIPLES IN THERAPEUTIC INOCULATION.

PART I.

PASTEUR was the first to think out the principles involved in Jennerian vaccination, to show how these could be applied to bacterial infections, and to conceive the idea of prophylactic inoculation against all infective diseases.

The tenets he laid down—let me in view of what is to follow speak of them as Code No. 1 or the original Pasteurian Code—may be set out somewhat as follows :—

CODE NO. 1.—THE ORIGINAL PASTEURIAN CODE.

1. The essential preliminary to any prophylactic procedure is to possess ourselves of the pathogenetic organism, or if this is as yet undiscovered of the virus that contains it, and to manufacture a vaccine from this.

2. The vaccine must consist of living germs; but these must, with a view to the risk that would attach to the employment of virulent material, be attenuated.

3. When an appropriately attenuated vaccine—that is, a vaccine which can be warranted to produce only a moderate clinical reaction—has been obtained, the exact quantum implanted will not be of material importance.

4. Bacterial vaccines should be implanted subcutaneously.

5. Vaccination is applicable only to the uninfected.

6. The protection conferred by the vaccine is always specific—in other words, protection is obtained only against the species of pathogenetic agent of which the vaccine consists.

7. Protection is obtained only after the lapse of ten or more days from the date of inoculation.

By putting into practice these principles—and they relate, as you see, only to prophylaxis—great and notable successes were achieved. In particular, Pasteur succeeded in protecting sheep and cattle against anthrax. Later Ferran, and after him Haffkine, employing a much more exact technique, applied the Pasteurian methods to man in their anti-cholera inoculations.

In the meanwhile, impelled to do so by that “pain in the mind” which is felt when one is appealed to and is powerless, Pasteur had addressed himself to another problem in connexion with immunisation—the problem as to whether it would, by any application of vaccination, be possible to arrest the development of rabies in a patient bitten by a rabid animal. Here, of course, his principle that vaccination was applicable only to the uninfected suggested that nothing could be done. But emotional tension is intolerant of any intellectual impasse, and its special function in such circumstances is to insist on a critical probing of subsisting rules, on a definition of all undefined or loosely-defined terms, and on a sharper focusing of all nebulous elements of thought. Here, therefore, it was bound inevitably to lead to a critical review of the principle that vaccination is applicable only to the uninfected, and to a general or sharper focusing of the terms “infected” and “uninfected.” And then it was discerned by Pasteur—and afterwards, of course, by all the world—that a patient in whom the virus of rabies has been implanted should, pending the development of events, be reckoned as uninfected; and, further, that if it takes for a virus much more than ten days, and for protection after vaccination only about ten days, to develop, the patient is within that period a perfectly suitable subject for preventive inoculation.

This forward step in thought, with which Pasteur broke away from what had been handed down and also from what he himself had formulated, bestowed upon us the preventive treatment of rabies. It was, as we shall see, destined to lead also to therapeutic inoculation.

A Supplementary Article of the Pasteurian Code.

We may now complete the Pasteurian Code as set out above by adding to it this supplementary item.

8. Vaccination may be resorted to in the incubation period of a disease, provided that that incubation period has still more than ten days to run.

That brings to a close the great opening chapter of scientific immunisation—the chapter contributed by Pasteur.

THE NEXT ADVANCE IN IMMUNISATION— ANTI-TYPHOID INOCULATION.

We have seen in the foregoing that emotional tension conducts to a closer scrutiny and sharper focusing of ideas, and that it is by the clarification which results from such closer focusing that problems are resolved. This holds true over the entire domain of knowledge, and is again exemplified in connexion with the next two advances in the field of immunisation—those made in connexion with anti-typoid inoculation. These improvements were (*a*) the substitution of dead for living vaccines, and (*b*) the preliminary gauging of the efficacy of prophylactic procedure by measurement of the anti-bacterial elements produced in the blood. Of these forward steps, the first was taken because of hesitation to risk inoculation with living typhoid bacilli; the second, from unwillingness to launch out with a vaccine of dead microbes without first obtaining proof of its efficacy.

The general progress of knowledge had already furnished certain facts and general inferences which bore on these issues. To begin with the conception that the antigens required were the metabolic products elaborated by microbes growing in the organism was becoming discredited, and was being gradually replaced by the conception that the antigens required were elements derived from the bacterial protoplasm. Again, the general principle—enunciated by Ehrlich in the apophthegm *corpora non agunt nisi soluta* was silently pointing to the conclusion that in vaccines the living or dead germs, as such, could not be the actual antigens; that these must be dissolved elements; and that the microbes in vaccines, whether they were dead or alive, could in reality be only mother substances of antigens. Lastly came the important fact that Pfeiffer had, by the incorporation of dead typhoid microbes into man, obtained a production of agglutinating substances. In consequence attention in the first anti-typoid inoculations was focused upon the production of agglutinins.¹ But the measurement of these showed very little more than that an immunisation response was obtained by the incorporation of dead microbes. Measurements of the bactericidal power of the blood² were then undertaken; and it was these that brought illumination. They established that when quanta of typhoid vaccine which produce moderately severe constitutional disturbance are incorporated, inoculation is followed first by a negative phase, in which the bactericidal power of the blood is reduced, and then by a positive phase in which the bactericidal power may be increased as much as 1000-fold; further, that when doses which produce very severe constitutional disturbance are employed the negative phase is protracted, and in

¹ Wright: THE LANCET, Sept. 19th, 1896; Wright and Semple: Vaccination against Typhoid Fever, B.M.J., Jan. 30th, 1897.

² Wright: Changes Effected by Anti-typoid Inoculation in the Bactericidal Power of the Blood, with Remarks on the Probable Significance of these Changes, THE LANCET, Sept. 14th, 1901.

some cases perhaps indefinitely protracted; and lastly, that when doses which produce only trifling constitutional disturbance are employed a positive phase is obtained without the intervention of any negative phase, and the bactericidal power of the blood is already after an interval of 24 hours very considerably increased.

These facts supply the principles which everywhere regulate—or perhaps it would be safer to say should everywhere regulate—anti-typhoid, anti-cholera, anti-pneumonia, anti-plague, and all other prophylactic inoculations.

Again, the observation that protective substances can be made to appear in the blood within 24 hours from the incorporation of a vaccine was one which carried consequences. It was deducible from this that the procedure of inoculation in the incubation period cannot be applicable only to rabies with its average incubation period of many months, but must, in theory, be applicable to typhoid and Malta fever with their incubation periods of a couple of weeks, and even—as contended by Haftkine—to plague with its incubation period of only a couple of days. But the full scope of application of the facts brought to light in connexion with anti-typhoid inoculation did not appear until the concept of an incubation period had been sharply focused, and until this had been seen to import a period when the microbe is cultivating itself in the body, not broadcast but confined in one region. Once that fact is regarded, there is, as you will see, no getting away from the conclusion that the successful event of the Pasteurian preventive treatment in rabies, and, it may be added, the occasional successful event of anti-plague inoculation administered in the incubation period of plague, must be due to immunising responses successfully evoked by the vaccine in the still uninfected regions of the body. That idea—the idea that the uninfected and still inactive regions of the body can by applying the stimulus of a vaccine be made to bring succour to the infected regions was, as you know, the mother idea of vaccine-therapy.

Now let me—for I shall want this as a point of departure for setting out my new matter—formulate in the form of a code the principles we have just been considering. The code now in question—for convenience of reference I call it Code No. 2—has been gradually elaborated in the Inoculation Department of St. Mary's Hospital. You will observe that here only the first, fourth, and seventh tenets have been taken over practically unmodified from Pasteur.

CODE NO. 2.—FOUNDED UPON A STUDY OF THE BACTERICIDAL AND OPSONIC CHANGES PRODUCED IN THE BLOOD BY THE INOCULATION OF VACCINES INTO THE PATIENT.

1. The essential preliminary to all immunisation procedures is to possess ourselves of the microbe of the disease, or failing that of its virus, and to employ this as material for the manufacture of the vaccine. And here it may be parenthetically pointed out that inasmuch as in prophylactic inoculation the vaccines are stock vaccines and give good results it cannot in any form of inoculation be theoretically essential to employ vaccines made directly from the patient.

2. Vaccines should in all cases where the microbe can be cultivated outside the body consist of sterilised cultures.

3. Vaccines may be turned to account in a variety of different ways. They may be employed not only for prophylaxis but also for preventive treatment in the incubation period of general infections. Again, they may be therapeutically employed in all localised infections other than those complicated by pyrexia and heavy and frequent auto-inoculations. In this last class of infections, and also in those septicæmic processes in which bacterial toxins in large quantity are circulating in the blood, vaccines are contra-indicated.

4. Bacterial vaccines should be incorporated hypodermically.

5. The quantum of vaccine incorporated is of supreme importance; it affects both the kind of response and the rate at which protective substances appear. With only small doses of vaccine, or comparatively light auto-inoculations, a positive phase—or as it may be better called, an epi-phylactic or immunising effect—may be registered in less than 24 hours after the incorporation of the vaccine. A similar but evanescent increase, known as the false rise,

may be registered within a couple of hours after larger doses of vaccine and heavier auto-inoculations. These larger inoculations of vaccine and heavier auto-inoculations produce after that very fleeting positive phase a negative phase or—for these are better terms—an apophylactic or de-immunising effect. And this effect is greater and lasts longer the larger the quantum of antigen carried into the blood.

6. In correspondence with the above the following rules of dosage may be laid down. In prophylactic operations undertaken in uninjected surroundings the dose should be that which evokes the optimum epi-phylactic response, and it is for the attainment of that end permissible to employ doses which produce a temporary negative phase. When inoculating prophylactically in the presence of an epidemic, or in the incubation period of a general infection, and generally in the treatment of localised infections, reduced doses should be employed in order to avoid the constitutional disturbance and temporary aggravation of symptoms and dispersal of microbes in the organism. Reduced doses should also be employed where the chief matter of concern is to obtain with promptitude some clinical improvement.

All these rules can be summarised into one general principle—that the dose of vaccine incorporated should depend on the patient's being infected or uninjected, and that where he is infected the dose should stand in inverse relation to the volume of his infection.

7. The anti-bacterial substances elaborated in response to inoculation operate specifically upon the variety of microbe which has furnished the vaccine, but it is possible that in addition some collateral immunisation is achieved.

I cannot here attempt to give even a brief summary of what has been achieved by the following out of this newer code. To do so I should have to chronicle all that has in the last 20 years been achieved by prophylactic and therapeutic inoculation in connexion with bacterial disease. Instead of attempting that, let me fix your attention on the fact that, except in a few isolated instances, therapeutic inoculation has proved unavailing in pyrexial phthisis and in septicæmia. And I may again point out that it is in the nature of things that the failures here in question should evoke in some of those who have had over and over again to witness them the same emotional tension and the same closer focusing of ideas which led to Jennerian vaccination, to Pasteur's thinking out the principles of immunisation, to the extension of prophylactic treatment to human diseases, to the Pasteurian preventive treatment of rabies, to the treatment of diphtheria by antitoxins, to vaccine-therapy, and to therapeutic advances along other lines. All this is, as you have discerned, only a prelude to setting out to you the outcome of an inquisition into those universally accepted principles which have hitherto guided vaccine-therapy, and to telling you of certain new principles which have in the last few years gradually emerged.

I have here to choose between two different modes of exposition. I could start by describing the new technical procedures which have been devised, could then, in connexion with each method, tell you the new facts it has furnished, and could then leave it to you to make at the end your own generalisations. That would be like leading you along a number of new paths, showing you hurriedly a portion of what could be seen from each, and leaving it to you to build up from those glimpses a general map of the country. The alternative, and proper, method will be for me, who have been every day engaged in exploring these paths, to give you at the start the general scheme of the landscape and then afterwards to describe to you the methods of exploration and tell what each of these has yielded.

The new principles which have emerged will best be set out in the form of yet another code. This will be Code No. 3, and I would wish you, when taken aback at its subversive character, to observe that there is here a going back, not upon anything directly established by experiment, but only upon dogmata built upon a foundation of uncoercive inferences. I have in view here not only the tenets of the Pasteurian code, but also the doctrine of phagocytosis with its implication that leucocytes can kill microbes only by phagocytosis. I wish you also to bear in mind that

the code here brought forward rests almost exclusively on experiments with the staphylococcus and streptococcus, that up to the present it has been put into actual operation almost exclusively in the treatment of infections by those microbes, and that the question of its application to prophylactic procedures is yet quite unstudied.

CODE NO. 3.—FOUNDED UPON A MORE DETAILED STUDY OF THE CHANGES PRODUCED IN THE BLOOD BY INOCULATION OF VACCINES IN VIVO AND IN VITRO.

1. While the nature of the infecting microbe should in every case be ascertained, it is not theoretically necessary that the vaccine employed in treatment or arrest of the incubating infection should be derived from the species of microbe which causes the disease. Sufficient justification for recourse to a particular vaccine is afforded when that vaccine has been shown to increase the antibacterial substances which operate upon the infecting microbe.

2. When vaccines in appropriate doses are added to the blood, whether in vivo or in vitro, instantaneous epiphytic response is evoked, and the maximal response may be expected after only very short delay.

3. The epiphytic response here in question consists in an extrusion of opsonic and bactericidal elements from the leucocytes. And it is mainly by this ectocytic chemical action, and only to an insignificant extent by phagocytosis and internal digestion, that the bactericidal action of the leucocytes is exerted.

4. The antibacterial substances here in question are polytropic—in other words, they operate not only upon homologous but also upon quite unrelated species of microbes.

5. Where the effective dose of vaccine for intravenous application has been ascertained, this method of administration is from considerations of certainty and rapidity of therapeutic action to be preferred to subcutaneous inoculation.

6. In septicæmias and other heavy bacterial infections the patient's leucocytes lose their power of responding to vaccines. In such cases it is essential before inoculating to satisfy one's self that the patient's blood still retains its power of epiphytic response.

7. Where by reason of the poisoning of leucocytes active immunisation by means of vaccines is ruled out, the method of immuno-transfusion should be resorted to—in other words, healthy human blood which has made proper epiphytic response should be incorporated.

Having now formulated the new principles arrived at, I must next show the data upon which these are founded. Those data were, as you will understand, obtained by adding measured quanta of vaccines or, as the case may be, living microbes, to the blood as a whole or to its separate elements. Let me begin with a conspectus of the methods of blood examination which have here come into application.

CONSPETCUS OF METHODS OF BLOOD TESTING.

I. Whole Blood.

- (1) Measurement of haemobactericidal power.
 - (Bactericidal effect of the leucocytes and serum acting in conjunction.)
 - Implanting and
explanting.
 - Implanting and
inculturing.
 - With defibrinated blood.
 - With washed leucocytes and serum (reconstituted blood).
- (2) Measurement of phagocytic power.

II. Serum.

- (1) Measurement of serobactericidal power.
 - Implanting and
explanting.
 - Implanting and
inculturing.
- (2) Measurement of opsonic power.

III. Leucocytes.

- (1) Measurement of phagocytic efficiency.
- (2) Measurement of spontaneous emigration and chemotactic reactions.
- (3) Measurement of ectocytobactericidal power (i.e., power of killing extracellularly).
- (4) Measurement of opsonodoteric power (i.e., power of furnishing opsonins).

IV. Combined measurement of—

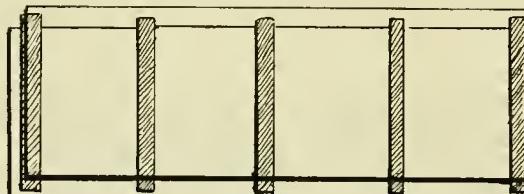
Phagocytic Power, Opsonic Power, and Phagocytic Efficiency of the Leucocytes.—*Chiastic procedure.*

A few words of general introduction and, in the case of those methods which have been newly devised, a

brief description will be required. Let me deal first with the measurement of the bactericidal power of the whole blood.

Methods of Measuring the Haemobactericidal Action.—This can be measured (1) by the *implanting and explanting procedure*, i.e., by bringing together measured volumes of defibrinated blood and graduated dilutions of a bacterial culture, and then after a fixed interval diluting with nutrient broth, and then either growing the surviving microbes in the broth-diluted blood, or explanting on to an agar surface. An alternative method of measuring the bactericidal power of the whole blood—available only when dealing with serophytic microbes—is (2) the *implanting and endo- or in-culturing procedure*. The principle of the latter method is to implant graduated quanta of microbes into the blood; to introduce the implanted blood into very fine capillary tubes, or, better, into "slide cells"; and to incubate, so as to let such microbes as survive grow out into colonies.

FIG. 1.



Slide cell ready for the reception of the implanted blood.

Slide Cells.—These are made from ordinary microscopic slides. We take a sterilised slide, place it with its convex side turned uppermost on the bench, and then provide confining walls for four compartments by taking strips of paper dipped in very hot vaseline, and laying these down at both ends and at equal intermediate distances along the slide. We then take another slide, hold it in the flame, concave side downwards, and then immediately press it down upon the vaselined strips. The paper employed for these strips must be of standard thickness so as to give blood films of appropriate and uniform depth. If the blood film is too thick the growing colonies are concealed, and if it is too thin the leucocytes are too remote from the implanted microbes—moreover, there is not a sufficient depth of red corpuscles to show up the colonies to advantage. A convenient depth of film is obtained by using for our strips a paper about $1/300$ inch (1/12th mm.) in thickness. The detail of the procedure for measuring the haemobactericidal power in slide cells is as follows: We begin by making a series of progressive dilutions of our bacterial culture. Then we measure out a series of 50 c.m.m. volumes of defibrinated blood and introduce into each of these (from a calibrated capillary pipette drawn out at the extremity into a fine point) 2-5 c.m.m. of one of the dilutions of our culture. Then after thorough mixing we introduce the implanted blood into one of the compartments of our slide cell. In preparation for this, the edges of the slide cell are re-sterilised by passing them through the flame, and the upper slide is pushed back a little so as to leave a projecting lower lip upon which to rest the point of the pipette which purveys the blood. When the compartments of the slide cell have been filled in with the allocated volumes of blood, the upper slide is brought back into position and the ends and sides are carefully sealed up by brushing them over with very hot melted paraffin wax. After 24 hours' incubation the staphylococcus colonies show up against the scarlet background of the blood by a surrounding dark purple discolouration. Then after a few more days this purple fades out and each colony is represented by a small bleached patch (Figs. 2, A and B). When instead of implanting into defibrinated blood we implant into a blood which coagulates in the slide cell, an additional feature arrests the attention. Each colony is encompassed by a ring. It is just as if we had taken an agar plate upon which staphylococcus colonies are growing, and a cork borer of much greater diameter than these, and had then, centring upon the colonies, cut into the medium and then withdrawn our cutting implement. It may be taken that the rings which encircle the staphylococcus colonies in the blood delimit the regions in which the fibrinous network has been digested by tryptic ferment generated either by the microbes or by the leucocytes which have gathered round them.

Reverting to the more general consideration of the measurement of haemobactericidal power, it will be well before passing on to appreciate that the

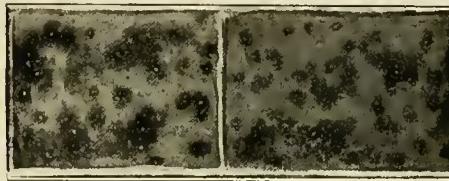
opportunity for phagocytosis afforded in the quiescent blood *in vitro* is certainly much greater than that afforded in the circulation. In that swift torrent the leucocytes have as little opportunity for ingestion as would fish if, instead of facing upstream, or swimming down faster than the current, they were only passively swept along side by side with all other flotsam. In the tranquil conditions provided in *vitro* the leucocytes will be able to congregate round their bacterial prey.

FIG. 2.—A.



Normal blood implanted with staphylococcus, showing the colonies which appear after 24 hours' incubation.

B.



Blood from a patient with streptococcal septicemia implanted and cultivated exactly as above.

Methods of Measuring the Phagocytic Power of the Blood.—We may here add one volume of the microbial suspension to two volumes of desibrinated blood³—or, following out the opsonic technique more closely, we may take one volume of washed corpuscles from the blood that is to be tested, one volume of serum, and add one volume of the microbial suspension.

Methods of Measuring the Sero-Bactericidal Power.—Here, as in connexion with the whole blood, we have two methods at our disposal. We can, using the procedures described above in connexion with blood, implant and then explant. When dealing with sero-phyltic microbes, in particular with the staphylococcus, we can also implant and inculture, filling our serum into fine capillary tubes and incubating in these.⁴ (Fig. 3.) By this method we obtain, as is seen in the figure, quite compact and separate colonies. The number of these will, of course, correspond, in the case of a non-bactericidal serum, to the number of microbes implanted; and in the case of a bactericidal serum to the number of microbes left alive. We can accordingly by this procedure compare the bactericidal potency of two sera, and we can for further control in such measurements count also the colonies which develop in nutrient broth. But in the ease of broth we must, because of the diffuseness of the colonies, limit ourselves to implantations of less than 100 microbes per 50 c.mm. volume, and must employ capillary tubes of such fineness that a 50 c.mm. volume may occupy a length of not less than 25 to 50 cm.; and above all, we must very carefully avoid all mechanical disturbances.

EXPERIMENTS : GRADUATED IMPLANTATIONS OF LIVING MICROBES INTO BLOOD IN VITRO.

Inculting Experiments.—We may now, leaving till later what requires to be said about the remaining methods of blood testing, study what happens when we make graduated implantations of living microbes

into blood. And it will here, as in all experimentation, be well to visualise and formulate quite clearly in advance what we expect will happen; for then both such results which conform exactly, and those which are at variance with expectation, will engrave themselves more deeply upon the mind.

Here, if anyone were required to say *a priori* what would follow upon making graduated implantations of living microbes into blood, he would assuredly say that as fewer and fewer microbes were added to blood a larger and larger percentage, and after a certain point a full 100 per cent. of the added microbes would be killed. Similarly he would predict of such graduated implantations that as more microbes were added the percentage of survivors would go up in a perfectly regular manner. The next step will be to see what does actually happen. Here a distinction has to be made between blood and blood. And I would ask you to let me tell you first what happens when I take my own blood and implant into it graduated quanta of microbes. I begin with my own blood because it so happens that this, by virtue of its having a subnormal number of leucocytes, and serum which has very little bactericidal action upon the staphylococcus, furnishes a specially favourable object for the experiments here in view. One of many dozens of quite conformable experiments made with the blood is set out in tabular form (Table I.). This particular experiment was carried out in duplicate by separate observers. The results of both workers are given.

It will be seen that we have here, with implantations of 10,000 to 150 staphylococci per c.cm., an average killing of 78·8 and 76·3 per cent. respectively; and with implantations below 150 staphylococci per c.cm. no killing at all—in other words with moderate implantations an average survival of 22 per cent., and with quite small implantations a survival of a full 100 per cent. These figures, I think, tell their tale quite plainly. Their import, as I read it, is that we have in the blood an apparatus for primary and an apparatus for secondary defence—a phylactic and an epiphylactic machinery. The former—making use, we may take it, of phagoeytic action, and where the serum is naturally bactericidal, also of sero-bactericidal action—would deal (according to circumstances more or less effectively) with small bacterial implantations and septicæmic sub-infections. The machinery for epiphylactic defence—making use, as we shall see, of anti-bacterial elements given out by the leucocytes when these are incited by antigen—would deal with larger bacterial implantations and graver blood infections.

³ Wright and Colebrook: *Technique of the Teat and Capillary Tube*, second edition, Constable, 1921.

⁴ The method of culture in capillary tubes was employed nearly 50 years ago by Salomonsen for the isolation of microbes from putrid blood.

FIG. 3.—Measurement of sero-bactericidal power. Staphylococci implanted into serum growing out into discrete colonies.

Interpreting now in the light furnished by this conception, the results set out in Table I., we see that in this blood the machinery of primary defence is quite singularly inefficient, while that of secondary defence is far more efficient. The blood, implanted

TABLE I.

Implantation of graduated dilutions of staphylococcus culture into 40 c.mm. volumes of A. E. W.'s defibrinated blood. The figures represent the number of staphylococcus colonies which developed after 24 hours' incubation in slide cells.

Implantation per c.c.m. of blood.	Observer, A. E. W.			Observer, L. C.		
	Number of microbes im- planted (I.) and colonies found (F.) in 40 c.mm. of blood.	Per- centage of survivors.	Implantation per c.c.m. of blood.	Number of microbes im- planted (I.) and colonies found (F.) in 40 c.mm. of blood.	Per- centage of survivors.	
8400	336	64	19	9600	384	74
4200	168	28	17	4800	192	44
2100	84	24	28	2400	96	27
1050	42	7·5	18	1200	48	13
525	21	11·5	55	600	24	15
262	10½	5	50	300	12	6
131	5	5		150	6	5
66	3	7		75	3	5
33	1·5	1·5		38	1·5	2
—	671	153	22·8	—	766	191
						24·9

It may be added here that precisely similar results are obtained when instead of, as in the tabulated experiments, implanting progressive dilutions of a bacterial suspension into separate samples of blood, we modify the procedure either (*a*) by implanting heavily into a volume of blood and then progressively diluting down this implanted blood with unimplanted, sampling it after each dilution; or (*b*) by making repeated small implantations into one and the same volume of blood, sampling it after each successive implantation.

with 33 microbes per c.cm., cannot kill one; when implanted with 10,000 per c.cm. it can kill 2000. This increased efficiency plainly indicates that an *epiphylactic response* has been evoked; and whenever we speak of the bactericidal action of the blood we must distinguish sharply between what the blood does where *epiphylactic response* is evoked and what it does where such a response is not evoked; and we should, when dealing with microbial implantations and infections, distinguish between a sub-vaccinating, a vaccinating, and a critical or optimum vaccinating dose.

But to interpret and generalise safely, we must regard not only bloods which have a feeble, but also those which have a powerful machinery of phylactic defence. When we take a blood which has a full complement of leucocytes, and a serum which has an appreciable bactericidal power, and make into this graduated implantations of staphylococcus, quanta up to 1000 and more per c.cm. are killed without residue; and with heavier implantations we have incomplete destruction—with (for this would seem to be the rule) first an increasing, then a diminishing, and after that again an increasing, percentage of survivors. With implantations of 10,000 to 20,000 staphylococci (these would, in case of powerfully bactericidal bloods, seem to be optimum implantations) we may have as few as 1 to 2 per cent. of survivors.

It will, on reflection upon the technical aspect of the question, be realised that with slide cells clear demonstration of *epiphylactic response* will, in the case of these powerfully bactericidal bloods, be difficult. A preliminary difficulty is created by the circumstance that with the heavier implantations which are here necessary accurate enumeration of the colonies becomes (owing to their crowding) impossible. A second difficulty is created by the fact that when we have instead of a nearly zero base line, a high base line of phylactic power, *epiphylactic response* can no longer show up clearly. And yet a third difficulty is created by the fact that instead of obtaining, as before, with vaccinating implantations, a bactericidal effect due wholly to *epiphylactic response*, we obtain, where the primary apparatus of defence is efficient, a result which is due in part to the factor of phylactic and in part to the factor of *epiphylactic action*.

These difficulties can be diminished by reducing the phagocytic power of these powerfully bactericidal bloods. Instead of working with the normal proportion of serum to corpuscles which obtain in defibrinated blood, we can by diluting down the blood with additional serum, work with an increased proportion of serum and a diminished proportion of leucocytes. Or, again, we can, by incubating our defibrinated blood for half an hour in fine capillary tubes, deplete it of leucocytes. Or finally, by using a combination of these methods we can obtain a blood which is both impoverished in leucocytes and diluted with serum. Such a blood will still, in that its serum exerts a bactericidal action on staphylococcus, differ from a blood of feeble phylactic power. But it will, in despite of that, behave very similarly. Small implantations will no longer be killed off without residue, and we obtain with geometrically diminishing implantations, a characteristically prolonged cauda of survivors.

Fixing our attention upon this cauda, let me explain to you how we can, where we have before us a table which sets out the results obtained from geometrically diminishing implantations of microbes, tell whether *epiphylactic response* has or has not been evoked. Here I would beg you—for this will greatly facilitate my exposition—to let me call the first figure in any column of implantations or results the *caput*, and all the figures which follow, the *cauda*. You will now on turning to Table I. and looking at the column of implantations see that the number of microbes in the *caput* must always exceed by a fraction (a fraction which corresponds to the number of dilutions) the total of microbes in the *cauda*. If now in the column of results we find the number of colonies in the *caput* sensibly the same as that in the *cauda*, the conclusion will clearly be that the bactericidal efficiency of the blood does not vary with the magnitude of the implantation. If the colonies in the *caput* much outnumber those in the *cauda*, the inference is that the bactericidal efficiency of the blood increases as the implantation is reduced. (Let it be remarked that it would inevitably do so if the serum exerts a bactericidal action.) But if the colonies in the *caput* are definitely outnumbered by those in the *cauda* we may—provided

TABLE II.

Experiment showing that when geometrically diminishing numbers of microbes are implanted into a powerfully bactericidal blood, and into the same blood diluted with serum, the serum-diluted bloods give a longer cauda of colonies, and that in the diluted bloods the cauda furnishes more colonies than the *caput*.

Number of living staphy- lococci im- planted into the 50 c.mm. samples of blood.	Number of colonies of staphylococcus which developed in—		
	Un- diluted blood.	Blood diluted—	
		With half its bulk of serum.	With its own bulk of serum.
100	1	3	4
50	0	1	3 (?)
25	0	1	1
12	1	0	1
6	0	0	0

always that the microbes in the first blood sample are not so numerous as to interfere with each other's development—confidently conclude that the bactericidal efficacy of the blood increases as the implantation is increased; in other words, that *epiphylactic response* has been evoked. And by going down the column of results in a methodical manner, comparing the number of colonies in each successive blood sample with the total for the two or three samples next in series, it will be possible to detect the point at which *epiphylactic response* comes in.

Employing these criteria in connexion with the results obtained from a powerfully bactericidal blood diluted down with serum, we see that evidence of *epiphylactic response* is here obtained. (Table II.)

Having now seen that bloods of powerful phylactic power when diluted respond, after the manner of undiluted bloods of feeble phylactic power, to larger bacterial implantations by increased bactericidal action (and all our other methods of testing epiphylactic response will bring confirmation of that) we shall, perhaps, do well before proceeding further to make a general survey of the field of bacterial infections, and see whether the doctrine of phylactic and epiphylactic defence enunciated above accords with the events which come every day under clinical observation.

Three types of cases should be called to mind :—

1. The type of infection (exemplified by streptococcal endocarditis) with for a long time only very few microbes in the blood, with only moderate pyrexia, comparatively little constitutional disturbance, and a protracted and almost always fatal course. These cases would well be explained by assuming that the machinery of first defence has failed to do its office and that the epiphylactic machinery has never been called into action.

2. The type of case (exemplified by croupous pneumonia) with from the outset an intense infection, high temperature, and heavy bacterial intoxication, and a course which very rapidly terminates either in death or in recovery by crisis. The cases which recover would be well explained by assuming that the epiphylactic machinery is here effectively, though tardily, brought into operation; those cases which terminate fatally, by supposing the epiphylactic machinery to have been put out of action by overwhelming bacterial intoxication.

3. The type of infection where we have either a regular hectic temperature, as in phthisis and locked-up suppuration, or else such steep ascents and descents of temperature as accompany the rigors of acute streptococcus septicæmias. Here we may assume—and such opsonic and bactericidal observations as are to hand are in accord with this—that whenever bacterial poisons in sufficiency are conveyed into, or generated in the blood, the machinery of epiphylactic defence is with results that are for the nonce satisfactory, called into action.

When once we survey the phenomena of infection from the point of outlook here suggested, the question arises in the mind whether in all these cases it might not be possible to bring the machinery of epiphylactic defence into effective operation by a direct introduction of vaccine into the blood. The proper dose to inoculate here would, of course, be that quantum (I might, perhaps, call it the critical dose) which would, taken together with the bacterial elements already in circulation, evoke in the patient's blood a maximum response. If the epiphylactic response to infection could in this way be expedited and reinforced, three advantages would be gained. First, the poisoning of the leucocytes—poisoning which upsets the machinery of both phagocytic and epiphylactic defence—would be avoided. Secondly, the patient would be spared that intense systematic poisoning which may bring him near to death before the life and death struggle with his infection even begins. And thirdly, the patient would be spared all that grave damage which is inflicted on his tissues at the site of infection.

IMPLANTATIONS OF DEAD AND LIVING MICROBES INTO THE BLOOD IN VITRO.

Inculcating Experiments.—Coming back to our experiments, we may consider next the effect of introducing into the blood instead of only living microbes a proportion of dead and a proportion of living microbes: (1) The dead may be implanted into the blood first and afterwards the living. That will be comparable to prophylactic inoculation undertaken with a vaccine of dead microbes, and followed by either a test implantation of living microbes or exposure to infection. (2) Or again the dead and the living microbes may be implanted into the blood simultaneously. (3) Or lastly—and this will be analogous to inoculation in the incubation period and to vaccine-therapy—the living microbes may be implanted first and afterwards the dead.

Let me put off for a moment telling you of the results obtained. For we must first clarify our ideas and see whether there is really a definite line of division between vaccinating and assaying, and ask

ourselves whether we have a perfectly clear idea as to what we mean by a vaccinating and an assaying or test dose. These will probably seem to you to be perfectly idle questionings, and you will, perhaps, think that they could be settled straight off, by defining the *vaccinating dose* as that which comes first in order of time, which normally consists of dead microbes, and which evokes the epiphylactic response; and the *assaying dose* as that which follows after, which consists of living microbes, and which finds out what epiphylactic effect has been obtained. In reality, however, these definitions do not go down to the root of things, and they regard only prophylactic immunisation.

When we pass to the case of inoculation in the incubation period and to vaccine-therapy, the terms vaccinating dose and test dose must, it will be allowed, be interpreted somewhat differently. The living microbes which have effected a lodgment in the body here officiate as the test dose, and the dead microbes which are subsequently inoculated, as the vaccinating dose. But here it may still be maintained that vaccinating and assaying are operations apart, and that here only the order of the doses has been changed. When, however, we come to the experiments in which an epiphylactic response is evoked by importing living microbes into the blood, it becomes plain that the distinction between vaccinating and assaying can no longer be sustained. For we have here not two separate and independent operations, but a single and indivisible vaccinating and assaying operation. And, of course, the same holds good of septicæmic infections. There the infecting microbes are the agents which produce, as the case may be, epi- or, as the case may be, apo-phylactic response, and they officiate at the same time as indicators of the changes effected.

There is still, in connexion with this subject, one further point that requires to be considered, and it is one which has already been adverted to. We saw in connexion with the discussion of dead and living vaccines that it is not the dead or living microbes as such, but soluble elements extracted from the bacteria which officiate as the antigen, and we further saw that such soluble elements are extracted irrespectively of the bacteria being living or dead. It follows that where (1) dead and living bacteria are implanted simultaneously or in succession into the blood, or (2) living microbes (in the form of abortive infections or test doses) are introduced into an organism already vaccinated, or (3) dead microbes are inoculated into an already infected organism, all the microbes, irrespectively of their being dead or alive, will make their contribution to the final result. And especially in connexion with therapeutic inoculation it will be important to observe that the really operative dose—the dose which, as the case may be, produces an epiphylactic or apophylactic effect—will in each case be the amount of antigen introduced in the vaccine, supplemented by that contributed by the infecting microbes.

This is the rationale of well-established rules—of the rule that in prophylactic operations we may, because here the active principle supplied from without will not be supplemented from within, employ a considerable dose of vaccine; and of the rule of therapeutic inoculation, that we must carefully consider the volume of infection, so as to introduce, as a larger quatum is supplied from within, a smaller quatum in the form of vaccine; and again of the rule that we should abstain from inoculation where a hypervaccinating dose is already circulating in the blood.

But though it is important to realise that when it is merely a question of furnishing antigen, dead microbes can take the place of living and vice versa, we must not allow ourselves to forget that, except only in the matter of furnishing antigens, the dead microbes which are introduced in vaccines stand on an absolutely different footing to the living microbes which produce the infection. The principles are in reality very simple. The dead microbes provide our therapeutic

agent, but the living decide the issue. Upon the dose of vaccine administered will depend the magnitude of the vaccinating response, but the outcome will, both in prophylactic and therapeutic procedures, depend upon the number of living microbes that have to be dealt with. We shall see that precisely the same holds also in vitro. It is essential to regulate the total of antigen which is brought into operation. But more important still is it to keep within certain limits the number of living microbes we implant in the assaying dose.

Three other points in connexion with experiments in which dead and living microbes are implanted into blood must also be kept in mind in connexion with every one of the experiments which I have cited and am about to cite. The first is that in what are designated living cultures there is always a variable and therefore unknown proportion of dead microbes. The second is that from the point of view of the evocation of physiological effects we must not range in the same category of efficacy all kinds of dead microbes or, as the case may be, all sorts of living microbes. To take the case of dead microbes, some like typhoid bacilli which have been killed at 56° C. readily dissolve in serum, and to a less extent in watery media. Others like typhoid bacilli, which have been heated above 72° C., are comparatively insoluble, and furnish vaccinating elements which are not only less in quantity, but also presumably less efficacious. And the third point to be borne in mind is that in vaccinating we are dealing, not as in ordinary chemical operations with reagents which are fully dissolved, but with substances which may sometimes fail to pass into solution, and may at other times pass into solution very slowly. It is in conformity with this that a dose of vaccine should sometimes give a blank result, and at other times, especially when inoculated intravenously, first a positive and afterwards a negative phase. Let me now show you the results of some experiments in which dead and living microbes were implanted into desiribinated blood in vitro.

EXPERIMENTS SHOWING THAT INCREASED HAEMOBACTERICIDAL EFFECT IS OBTAINED BY VACCINATING BLOOD IN VITRO.

Below are subjoined first the result of two experiments conducted on my own blood.

Effect of Implanting Dead and Living Microbes into Blood.

Here the dead and living of microbes were implanted simultaneously into 25 c.mm. volumes of blood and the implanted blood was afterwards filled into and incubated in fine capillary tubes.

Experiment 1.

Test dose.*	Vaccinating dose.†	Aggregated implantation.	Negative or positive result of culture.
Living staph. per c.c.m. Circ. 2,000	Dead staph. per c.c.m. Circ. 32,000	Circ. 34,000	+
"	"	"	+
"	16,000	18,000	+
"	"	"	+
"	8,000	10,000	+
"	"	"	+
"	5,000	7,000	0
"	"	"	0
"	4,000	6,000	+
"	"	"	+
"	2,000	4,000	+
"	"	"	+
"	Nil	2,000	+
"	"	"	+
"	"	"	+

Staph. = staphylococci.

* 1 wash of a dilute suspension of living staphylococcus.
† 1 wash or a multiple or submultiple of a sterilised 8-fold stronger staphylococcus suspension.

Details of the Bacterial Enumerations upon which the Figures in the Above Table are Based.—The staphylococcus suspension which furnished the test doses was further diluted 10-fold, and of this a wash or a submultiple of a wash was implanted and inculcuted in 25 c.mm. volumes of my serum.

1 wash gave 5 colonies.
1 " " 5 "
½ " " 3 "
¼ " " 1 colony.

There being thus in 1 wash of the diluted culture used for this enumeration an average of 5 microbes, there would be in 1 wash of the suspension which furnished the test dose 50 microbes. And this (seeing that 25 c.cm. of blood were in each case employed) works out as an implantation of 2000 (50×40) staphylococci per c.cm.

Experiment 2.

Here a precisely similar technique was employed.

Test dose.	Vaccinating dose.	Aggregate implantation per c.c.m.	Number of survivors per c.c.m.
Living staph. per c.c.m. Circ. 1,200	Dead staph. per c.c.m. Circ. 12,000	Circ. 13,200	363
"	"	"	
"	6,000	7,200	48
"	"	"	
"	4,000	5,200	180
"	"	"	
"	3,000	4,200	462
"	"	"	
"	Nil.	1,200	200
"	"	"	

Experiment 3 shows a quite dramatic result obtained with a blood which was *ab origine* one of great bactericidal power.

Experiment 3.

Here the vaccine of dead staphylococci was implanted in vitro into desiribinated blood. The vaccinated blood was kept on the bench and then after three-quarters of an hour graduated dilutions of living staphylococci were implanted—the volume of staphylococcus suspension being always 2.5 c.mm. in 50 e.mm. of blood. After implantation the blood was filled into slide cells and inculcated.

Variety of blood into which living microbes were implanted.	Number of microbes per cent. which developed from an implantation of—							
	20,000	10,000	5,000	2,500	1,250	600	300	150
Unvaccinated blood	7.4	5.2	6	5	16	6.6	0	0
Blood vaccinated with—								
12,000 staph. per c.c.m.	1.5	1.4	0.8	1.6	1.6	3.2	0	0
6,000 dead staph. per c.c.m.
3,000 dead staph. per c.c.m.	0.6	0.4	0.8	0.0	1.6	3.3	7	0
1,500 dead staph. per c.c.m.	6.1	1.6	5.2	8	1.6	3.2	0	0
	5	5.4	5.6	6	1.6	3.2	0	0

Details of the Bacterial Enumeration upon which the Figures in the Table are Founded.—Here in each case 2.5 c.mm. of the 32-, 64-, and 128-fold dilution of the bacterial suspension used for the strongest implantation was incorporated into 50 c.mm. of serum, and then inculcured. The number of colonies which developed in the serum implanted with the 32-, 64-, and 128-fold dilutions were respectively (the counting operations being conducted in duplicate) 31 and 30, 18 and 19, and 6 and 7. This (taking the first figures) works out as (30×20) an implantation of approximately 600 living staphylococci per c.c.m. in column 6 of the table.

Attention may here be directed to three points:—

1. All the vaccinated bloods kill here all along the line more microbes than the unvaccinated blood, the best results being in this particular case obtained with blood vaccinated with 6000 dead staphylococci. This blood in the case where 20,000 living staphylococci were implanted killed off per c.c.m. 1300 more staphylo-

cocci than the unvaccinated blood; and it killed off without residue 2500 as compared with 300 staphylococci.

2. The second point is one of general theoretical importance. When comparable numbers of microbes are employed, these being, in the one case, all derived from a living culture, and, in the other case, in part from a living and in part from a sterilised culture, better results are in every case obtained with the blend. Thus, for example, we have in column 1 with an aggregate implantation of 20,000 living staphylococci a percentage survival of 7·4, and in column 2 with an aggregate implantation of 22,000 staphylococci, consisting of 12,000 dead and 10,000 living, a percentage survival of 1·4; and again, we have in column 2, with an aggregate implantation of 10,000 staphylococci a percentage survival of 5·2, and in column 3, with an aggregate of 11,000 staphylococci, consisting of 6000 dead and 5000 living, a percentage survival of 0·8. It would thus seem—but one must be cautious in inference, because there are no cultures which contain only living microbes—that dead microbes are, micro-organism for micro-organism, more efficacious than living; and that vaccines of sterilised cultures should be preferred to living vaccines, not only because their use is unassociated with risk of infection, but also because they are superior in quality.

3. The third point is also of fundamental interest. And it is a point that has already been considered. When in our assaying operations we introduce microbes into the whole blood or into a serum or pus fluid which contains leucocytes, or even, as in the opsonic technique, into an artificial mixture of washed leucocytes and serum, the microbes we implant may, despite of our having implanted them solely for the purpose of testing, evoke (this will depend on their number) a secondary epiphytatic response which will give increased anti-bacterial power such as is presumably achieved when slighter infections supervene upon a prophylactic procedure. Or again, the microbes we implant for the purpose of testing may, like graver infections supervening upon a prophylactic procedure, get the better of the blood resistance, and eat up, or more than eat up, the surplus of anti-bacterial power furnished by the original vaccinating operation. It will, in view of these superadded effects, be very difficult to assess the actual achievement of the prior vaccinating operation. Apparently the best that can be done in the way of assessment will be to get the leucocytes out of the way before we undertake our tests, and content ourselves with measuring the bactericidal and opsonising power of the serum. Consideration of the effect produced by the intravenous inoculation of vaccines upon the haemobactericidal power may with advantage be postponed until the argument of this lecture has been a little further developed.

IMPLANTING AND EXPLANTING EXPERIMENTS.

Up to the present we have been studying the results obtained by implanting living microbes separately or associated with dead microbes into the blood and inculturing. This method measures the destructive power of leucocytes and blood fluids operating in conjunction. But while it tells us what number of microbes are killed it tells us nothing about the pace of destruction, and nothing even indirectly about the agency by which the microbes are destroyed. And again the inculturing method has a definitely limited range of utility. For when over, let us say, 60 colonies develop in 50 c.mm. of blood accurate enumeration begins to become difficult. The implanting and explanting method supplies what is wanted in these respects. It will be proper first of all to satisfy ourselves that the general results obtained by this method are conformable to those obtained by inculturing. And, inasmuch as we are here specially concerned with the verification of the proposition that epiphytatic response can be evoked in the blood *in vitro*, and is evoked only when we implant sufficiently heavily, it will be well to first study my blood whose feeble phylactic power makes it specially

well adapted for the institution of experiments on epiphytatic response.

In these implanting and explanting experiments implantations of 2·5 c.mm. volumes of graduated dilutions of a suspension of living staphylococci were made into 50 e.mm. volumes of A. E. W.'s desiribinated blood. Then samples were taken from each implanted blood, and these were first diluted 25-fold and then spread out over the surface of agar plates. The first samples were explanted instantaneously—instantaneously being here understood to mean instantaneously after the blood and microbes had been duly mixed—and the other samples after longer intervals. The implanted bloods were in each case kept not in the incubator but at the temperature of the air.

Experiment A.

Blood vol.	Number of living staphylococci implanted		Number of colonies which developed from that volume explanted after the stated interval.				Number of staphylococci implanted into the samples.	Number of colonies which developed out of those samples.
	per c.c.m. of blood.	2 c.mm. of blood.	0'	30'	60'	120'		
1	16,500	33	26	20	27	23	132	96
2	11,000	22	12	6	11	—	66	29
3	5,500	11	8	2	3	5	44	18
4	2,750	5·5	2	1	1	1	22	5
5	920	1·9	1	2	2	1·5	7·6	6·5
6	460	.9	1	0·5	0	1·5	3·6	3
7	230	.45	0	0	0	0·5	·45	0·5

Experiment B. (The same technique was employed.)

Blood vol.	Number of living staphylococci implanted per c.c.m. of blood.		Bactericidal effect in sample explanted immediately.		Bactericidal effect in the four samples explanted within three hours.	
	12,000	8,000	22 reduced to 8	90	184	15
2	8,000	15	" 10	60	42	5
3	4,000	7·5	0	22·5	reduced to 7	7
4	2,000	3·75	becomes 5	11·5		
5	800	2·25	2	7·75		8
6	516	1·5	" 2	4·5	14·5	5
7	270	0·75	" 0	2·25	becomes 3	3

Experiment C. (Again the same technique was employed.)

1	5,400	10·8 reduced to 5	43	57·5	(18)
2	1,800	3·6	1	14·5	7
3	360	0·75 becomes 1	3	4	3
4	170	0·25 "	0	1	0

It will be seen that the conclusions which were reached by the inculturing method are here all confirmed. We again arrive at the result that the particular type of blood here in question exerts no bactericidal effect when only small numbers of staphylococci (1000 per c.c.m. and fewer) are implanted, and that it exerts a very conspicuous effect when larger numbers of staphylococci are introduced. In the three experiments here set out there is with the smaller implantations no killing whatever, and with the larger implantations an average killing of 60 per cent.

Experiment D.

Here 7·5 c.mm. of an enumerated staphylococcus culture was implanted into 300 c.mm. of A. E. W.'s desiribinated blood and immediately afterwards a 30 e.mm. sample was explanted on to an agar plate. That done another 7·5 c.mm. of the staphylococcus suspension was implanted, and immediately another 30 c.mm. sample was explanted—the same procedure being followed throughout.

Number of staphylococci implanted per c.c.m. of blood.	Number implanted in the 30 c.c.m. of blood used for explantation.	Number of colonies which developed from that 30 c.c.m. of blood.	Percentage of survivors.
400	12	11	99
840	25	10	40
1,310	40	—	—
1,850	56	18	32
2,450	75	26	34
3,125	100	17	17
4,625	140	48	29
6,300	190	69	31
8,150	245	108	44
10,250	310	148	48

The figures for the implantations were obtained by enumerating the staphylococcus suspension in 50 c.mm. volumes of serum. An implantation of 2·5 c.mm. gave 44 colonies, one of 7·5 c.mm. 140 colonies, and one of 10 c.mm. 135 colonies, giving a total of 319 colonies in the 20 c.mm. of suspension implanted. This works out as 16 staphylococci in 1 c.mm., and 12 in 0·75 c.mm., the volume of the bacterial suspension in the 30 c.mm. of blood used for the first implantation.

Further, these experiments bring out—and this is their new contribution—that of the total of microbes killed by the blood the larger proportion (here two-thirds of those killed) are killed instantaneously.

Let me in connexion with this make clear the following points: (1) Subitaneous killing of microbes is obtained practically invariably. Where it fails to occur that may perhaps depend upon the antigen not being brought into solution. (2) The fact that microbes can be killed instantaneously by the evoking of epiphylactic response has, as we shall presently see, very important practical applications in connexion with the treatment of bacterial disease. (3) The fact that microbes are killed instantaneously and at ordinary temperatures throws important light on the mode of bacterial destruction and on the nature of epiphylactic response. If we had had to depend here only upon the inculting method we might perhaps have supposed that the implanting of a sufficient quantum of microbes led to an activation of the machinery of phagocytosis. But when we learn, as we do from these experiments, that microbes are killed instantaneously at temperatures at which phagocytosis begins only after very appreciable delay, we are compelled to conclude that subitaneous killing cannot be due to phagocytosis. It must therefore perforce be attributed to a bactericidal action of the serum.

The generalisation that subitaneous killing of microbes in the blood is due to a bactericidal action exerted by the serum furnishes in its turn a new articulating point upon which a further framework of generalisations can be assembled. From the fact that the blood can kill microbes instantaneously it can be inferred that a bactericidal serum also must be competent to kill instantaneously. Further, when we have realised that the serum receives, when we vaccinate the blood, a quite sudden access of bactericidal power, and when we reflect that where massed leucocytes washed free from serum are imposed upon agar implanted with staphylococci or streptococci, these⁵ last are killed extracellularly and by chemical agency, we are almost inevitably conducted to the conclusion that the bactericidal elements found in vaccinated bloods are derived from the leucocytes, and that leucocytes can, under the influence of a vaccinating

dose of microbes, instantaneously export their anti-bacterial elements into the surrounding blood fluids.

We shall, as we proceed, see all of these inductions confirmed by actual experiment. But here another issue must take precedence. I have already elsewhere⁶ shown that when epiphylactic response is evoked by adding vaccine to blood the serum becomes bactericidal, or as the case may be, more bactericidal, not only to the species of microbe which has furnished the vaccine, but also to many non-homologous microbes. This does not stand by itself. It would seem that all the antibacterial elements that are brought forth in the blood—and it will presently appear that opsonic substances also are generated—are polytropic.

It is unnecessary to say much in the way of general comment on the fact that the antibacterial substances here in question are polytropic. For while established notions prejudice us against the idea of vaccination conferring a non-specific protection, reflection puts out of court all alternative suggestions. In the first place, where the blood kills microbes instantaneously there is time for delivery but not for manufacture of anti-bacterial elements. And again, it is much more difficult to conceive that the leucocytes should have stored up in their protoplasm special monotropic bactericidal substances and opsonins for each and every description of microbe than to conceive of the leucocytes being furnished with a common store of polytropic antibacterial elements which could be brought into operation upon all microbial invaders without distinction of species.

And in this connexion something further may be added. When once we have pictured to ourselves that non-specific anti-bacterial substances can, under the stimulus of any antigen, be exported from leucocytes, it does not perhaps involve a much more adventurous flight of imagination to conceive that an effective vaccinating stimulus might be supplied to the leucocyte by chemical agents other than bacterial antigens. Certain clinical and also some experimentally established facts would very well harmonise with such a conception. Here would come the fact that emetin, though not directly noxious to the *Amoeba histolytica*, can rid the intestine of that infection. To this might be added the fact that salvarsan can in syphilis, complicated with a heavy streptococcal infection, very rapidly rid the body of the latter—a fact which should be brought into the same visual field with the observations of S. R. Douglas and L. Colebrook, which establish that after salvarsan and neosalvarsan injections the blood becomes for a period of a few hours bactericidal to streptococcus and staphylococcus. And again, there might be added the observations of Walbum,⁷ which show that the yield of diphtheria antitoxin and of agglutinins to the *Bacillus coli* can be very appreciably increased by the exhibition of mauganese chloride and other metallic salts.

⁵ Wright, Fleming, and Colebrook: Sterilisation of Wounds by Physiological Agency, THE LANCET, June 15th, 1918; Wright and Colebrook: Technique of the Teat and Capillary Tube, pp. 279-283.

⁶ Wright: Lessons of the War, THE LANCET, March 29th, 1919.

⁷ Communications de l'Institut Scro-thérapique de l'Etat Danois, tome xii., 1922.

PART II.

WE are now in a position to examine in more detail the nature of epiphylactic response. Certain very simple reconnoitring experiments give us our bearings. The experiments in question consist in killing the leucocytes, in some cases before, in other cases after, implanting microbes into the blood and seeing how far this killing of leucocytes affects bacterial destruction. An illustrative experiment is subjoined. Here before the microbes were implanted, the blood was heated to a temperature of 46° C.-48° C., this being a temperature which kills the leucocytes and leaves the serum, and if not kept up unduly long, also the microbes unaffected.

Here graduated implantations of staphylococcus were made by the "wash and after-wash" method into a series of volumes of A. E. W.'s blood, these volumes being drawn up one after the other into a long-stemmed capillary tube.

	Number of colonies which developed in vols.															Total in the last 12 vols.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
A { (1) ..	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B { (1) ..	+	+	+	3	3	2	1	1	0	1	1	1	0	0	0	13
	+	+	+	5	5	2	0	0	0	0	1	0	0	0	0	13

A = Unheated blood. B = Blood pre-heated to 48° C.

The sign + is employed to indicate that the colonies were too closely packed to allow of accurate enumeration.

It will be clear from this quite typical experiment that the killing of leucocytes makes a very profound difference. The pre-heating of the blood abolishes or nearly abolishes, in two several ways, the bactericidal power of the blood for staphylococci and streptococci. First, it puts an end to phagocytic action (in the case of my own blood that would apparently not count for much); and further (and this in all bloods counts for a great deal more) it abolishes also the power of epiphylactic response, the blood being then left with only such microbial powers as it possessed *ab origine*. With respect to the experiments in which the blood, after microbes have been implanted, is heated sufficiently to kill the leucocytes and not sufficiently to affect the serum or injure the implanted microbes, these experiments give, as you will expect to hear, results which are in absolute agreement with those obtained in implanting and explanting experiments. When we implant into a blood and then immediately apply a temperature of 46°-48° C. we obtain very much less growth than in an unheated blood. When the application of heat is postponed for a minute, appreciably more microbes are killed; and when the unimplanted blood is allowed to lie upon the bench for an hour or so before it is heated the cultural results are practically the same as in a blood left unheated.

EXPERIMENTS WHICH SHOW THAT WHEN EPIPHYLACTIC RESPONSE IS EVOKED IN THE BLOOD THE BACTERICIDAL POWER OF THE SERUM IS INCREASED.

Such experiments as those cited in which the blood is heated either before or after the implantation of microbes are, it must be remembered, only reconnoitring experiments. They do not make it absolutely certain that it is by the action of the leucocytes or almost exclusively by their action that staphylococci and streptococci are killed in the blood. And again, these heating experiments—and the same applies, of course, to all experiments made with the whole blood—tell us nothing except by way of indirect inference with regard to the manner in which the killing of the microbes is accomplished. To arrive at

certain knowledge we must experiment with each of the elements of the blood separately.

We may advantageously begin by satisfying ourselves that epiphylactic response goes hand in hand with a development of increased bactericidal power in the serum. As I have already elsewhere¹ shown that this holds true of human blood vaccinated in vitro, I may profitably here cite experiments of a different kind—experiments in which vaccines of dead microbes are inoculated intravenously into animals and man, and experiments in which the sera are tested immediately and at different time-intervals after inoculation.

EXPERIMENTS ON RABBITS.

Here a vaccine of dead microbes was inoculated intravenously, samples of blood were withdrawn before and at intervals after inoculation, and then similar implantations of living staphylococci were by the wash and after-wash method made into a sequence of 10 c.mm. volumes of each serum. The figures in the table show the number of staphylococci which grew out into colonies in the different sequences of 10 c.mm. volumes—the numbers being in each case expressed in terms of microbes per c.c.m. of serum.

Rabbit.	Microbes per c.c.m. which grew out into colonies in sera of bloods drawn off before and after injection of vaccine.						Additional number of microbes killed by the serum after injection of vaccine.	
	1 Before after	15 min. after	30 min. after	1 hr. after	3 hrs. after	6 hrs. after		
1. Inoculated with 20,000 staph. per c.c.m. of blood ..	5000	100	—	100	—	0	0	At least 5000 per c.c.m.
2. Inoculated as Rahhit 1	2200	1200	200	—	—	400	0	At least 2200 per c.c.m.
3. Inoculated with 14,000 strepto-spyrocytes per c.c.m. of blood ..	5000	500	0	—	0	—	0	At least 5000 per c.c.m.

Staph. = Staphylococci.

EXPERIMENTS ON MAN.

I. Here 250,000 dead streptococci—i.e., about 40 streptococci per c.c.m.—were injected intravenously into A. E. W.'s blood, samples of blood were drawn off before and after the injection, and the bactericidal powers of the sera were tested by implanting by the wash and after-wash method graduated doses of living staphylococci into a sequence of equal volumes of serum, filled in each case into a long capillary tube.

	Positive or negative result of culture in successive unit-vols. of serum.															Number of vols. which showed growth.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1. { (a) ..	+	+	+	+	+	+	+	+	?	?	+	+	+	+	+	13 { 14
{ (b) ..	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
2. { (a) ..	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	7 { 8
{ (b) ..	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	9
3. { (a) ..	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	6 { 6
{ (b) ..	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	7 { 6

1. Serum immediately before injection.

2. Serum 2 min. after injection.

3. Serum 15 min. after injection.

II. Here a dose of streptococcus vaccine equivalent to some 15 streptococci per c.c.m. of circulating blood was inoculated intravenously into a patient suffering from a streptococcus infection. Samples of blood were taken immediately before, immediately after, and 21 hours after the injection. The sera were tested by implanting with living staphylococci by the "wash and after-wash" method in each case into a sequence of one 20 c.m.m. and two 10 c.m.m. volumes of serum.

	Number of colonies which developed in—			
	Vol. 1.	Vol. 2.	Vol. 3.	In the 3 vols.
Serum of blood before inoculation (a)	11	1	1	13 ¹ 13·5
(b)	13	1	0	14 ¹
Serum of blood immediately after inoculation (a)	8	1	0	9 ¹
(b)	5	0	0	5 ¹ 7
Serum 24 hours after (a)	15	0	0	15 ¹
(b)	13	0	0	13 ¹ 14

The difference of 6·5 colonies in 40 c.m.m. of serum corresponds to a killing of 162 more staphylococci per c.c.m. of serum.

If we take into consideration only the first, more heavily implanted 20 c.m.m. volumes we have a difference of 5·5 colonies corresponding to a killing of 275 staphylococci per c.c.m.

III. Here a dose of vaccine corresponding to about 15 streptococci per c.c.m. of circulating blood was inoculated intravenously into a patient suffering from a heavy cold, followed by a further intravenous injection of 10 streptococci per c.c.m. of blood 21 hours after, when the cold was decidedly better.

The sera were tested by implanting 5 c.m.m. of a staphylococcus suspension into 100 c.m.m. volumes of serum.

August 23rd, 1922: Serum before inoc. furnishes 76 staph. colonies.

Serum immediately after inoc. furnishes 63 staph. colonies.

The difference of 13 colonies per 100 c.m.m. corresponds to a killing of 130 more staphylococci per c.c.m. of serum.

August 24th, 1922: The sera were here tested in the same way but, of course, with a different staphylococcus suspension. Serum immediately before inoc. furnishes 40 colonies.

" 24 hours after " " 30 "

The difference of 10 colonies in 100 c.m.m. corresponds to a killing of 100 more staphylococci per c.c.m.

IV. Intravenous inoculation of a streptococcus vaccine into a normal man.

May 10th, 1922: 500,000 dead streptococci were inoculated, and the sera were tested by implanting a 20 c.m.m. wash of a staphylococcus suspension in each case into three 20 c.m.m. volumes of serum.

The volumes of serum immediately before inoculation furnished—

36, 38, and 30 colonies—average 35.

Those of serum after inoculation furnished—

27, 34, and 29 colonies—average 30.

Those of serum 20 hours after inoculation furnished—

23 and 28 colonies—average 25.

May 15th, 1922: 800,000 dead streptococci were inoculated. The sera were tested as before.

The volumes of serum immediately before inoculation furnished—

13, 16, and 9 colonies—average 13.

The volumes of serum after inoculation furnished—

7 and 8 colonies—average 7·5.

The volumes of serum 24 hours after inoculation furnished—

16 and 18 colonies—average 17·5.

Calculation shows that the blood immediately after the first inoculation killed 250 more staphylococci per c.c.m., and 20 hours after 500 more per c.c.m.; and that it killed immediately after the second inoculation 275 more per c.c.m.

V. Intravenous inoculation of streptococcus vaccine into a normal man.

Dec. 29th, 1922: Sera from bloods drawn off before, 1½ hours after, and 5 hours after the inoculation of 160,000 dead staphylococci; 50 c.m.m. volumes of each serum were implanted with 2·5 c.m.m. of a 2700-fold dilution of a broth culture of staphylococcus.

Serum before inoculation furnished 47 colonies.

Serum 1½ hours after " " 21 "

Serum 5 hours after " " 42 "

Calculation here shows that the serum 1½ hours after inoculation kills 520 more staphylococci than before.

VI. Streptococcal endocarditis.

Sept. 16th, 1922: Sera from bloods drawn off before and immediately after an intravenous inoculation of 25,000 dead staphylococci. The technique employed and the staphylococcus suspensions employed were the same as in the last case.

Serum Before Inoculation.—Implanted with the more dilute suspension of staphylococcus furnishes 35 and 48 colonies (average 40·5); implanted with the more concentrated suspension 71 and 83 colonies (average 77).

Serum Immediately After Inoculation.—Implanted with the more dilute suspension furnishes 32 and 34 colonies (average 33); implanted with the more concentrated suspension 65 and 66 colonies (average 65·5).

This, taking again the figures for the heavier implantation, works out as an increased killing of 11·5 per 50 c.m.m. and 230 for 1 c.c.m. of serum.

EXPERIMENTS WHICH SHOW THAT THE VACCINATION OF BLOOD MAY INCREASE THE OPSONIC POWER OF SERUM AND ALSO THE PHAGOCYTIC POWER OF BLOOD.

Let me now go on to show that when epiphylactic response is evoked in the blood the opsonic power of the serum, and pari passu with this, if the phagocytic efficiency of the leucocytes is not reduced, the phagocytic power of the blood is increased.

Reference has already been made to the fact that the inoculation of the blood with bacterial vaccines increases the opsonic power of the serum. This development of opsonic power proceeds on quite similar lines to the development of bactericidal power. It manifests itself both *in vitro* and *in vivo*, and it begins practically immediately.

EXPERIMENTS IN WHICH VACCINES WERE INOCULATED INTRAVENOUSLY INTO RABBITS.

Rabbit 1.—Inoculated with 10 million of dead staphylococci (about 40,000 per c.c.m.). Opsonic power of serum—before inoculation 1; 3 minutes after, 2·9; 1 hour after, 2·3; 1½ hours after, 3·0.

Rabbit 2.—Inoculated with 5 million of dead staphylococci (about 20,000 per c.c.m.). Opsonic power of serum—before inoculation, 1; 3 minutes after, 1·2; 2½ hours after, 1·3.

EXPERIMENTS IN WHICH VACCINES WERE INOCULATED INTO HUMAN BLOOD IN VITRO AND IN VIVO.

I. Intravenous inoculation of about 40 streptococci per c.c.m. into A. E. W.'s blood. Blood 1 was drawn immediately before, blood 2 immediately after, and blood 3 15 minutes after inoculation. The opsonic power of the sera and the phagocytic efficiency of the leucocytes were then measured—the opsonic power by taking 1 volume of serum, 1 volume of the staphylococcus suspension, and 1 volume of washed leucocytes; the phagocytic efficiency by comparing the microbial intake obtained when the microbial suspension was used with the same sera and different leucocytes.

—	Staphylo-phago-cytie count.	Staphylo-opsonic index.	Average ingest with the 3 sera.	Phago-cytie efficiency.
Washed corps. of blood 1—				
+ serum 1 + staph.	3·4	1		
+	5·5	1·6		
+	6	1·8		
			5	1
Washed corps. of blood 3—				
+ serum 1 + staph.	3·5	1		
+	3·9	1·1		
+	4·8	1·4		
			4	0·8

I want to draw attention here to three points: To the fact that the opsonic index of the blood goes up instantly; to the fact that the phagocytic efficiency of the leucocytes goes down; and to the fact that the effect of the increased opsonic power is in this case partially masked by the decreased leucocytic efficiency. This comes out when we regard serum 3, and note that it gives with the corpuscles of blood 1 an average ingest of 6, and with the corpuscles of blood 3 an ingest of 4·8 only.

We shall presently see that the changes which occur in the blood in acute streptococcal infections follow this pattern except only in the respect that we have there usually a smaller rise in the opsonic index and a much profounder fall in the leucocytic efficiency.

In the next experiment (II.) it will be seen there is: 1. A notable increase of strepto-phagocytic power obtained with both varieties of vaccine. 2. Further, in each case when a certain dose is exceeded the phagocytic power begins again to decline. Other results show that this decline is probably due to the increased opsonic power of the serum being now counterbalanced by a poisoning of the leucocytes. 3. In this particular case a greater rise in the strepto-phagocytic power is obtained with the homologous vaccine. This might, of course, be due to the homologous character of the vaccine, but it might also be due to the dose of streptococcus having been

here more fortunately chosen, either from the point of view of its evoking a better opsonic response, or from the point of view of its being less toxic to leucocytes.

The same probably holds true of all the following experiments. In connexion with the sixth experiment of the series, it will, on comparing the phagocytic indices with the opsonic indices—i.e., on comparing the amount of phagocytosis obtained with the leucocytes of the vaccinated blood with that obtained with the leucocytes of the unvaccinated blood—be seen that increase in the opsonic power of the serum is to a large extent masked by the poisoning or exhaustion of the leucocytes.

II. Here, with a view to finding out what would be the better kind and optimum quantum of vaccine to add to a prospective donor's blood for the purposes of immunotransfusion, graduated additions of typhoid and streptococcus vaccines were made to his defibrinated blood in vitro. The samples of blood were incubated with the vaccine for one hour in vitro, and were then tested for phagocytic power by adding one volume of streptococcus suspension to two volumes of defibrinated blood.

—	Strepto-phagocytic index.	—	Strepto-phagocytic index.
Unvaccinated blood ..	1	Unvaccinated blood ..	1
Blood vaccinated with typhoid bacilli:		Blood vaccinated with streptococci	
1,000 per c.c.m. ..	1·7	20 per c.c.m. ..	3·6
2,000 ..	2	40 ..	4
4,000 ..	1·35	80 ..	2
8,000 ..	1·75	200 ..	2·3

III. Here a number of volumes of A. E. W.'s defibrinated blood were inoculated by the wash method with graduated dilutions of staphylococcus vaccine. After incubation for half an hour at 37°C. their phagocytic power was tested with a staphylococcus suspension containing about 200,000,000 staphylococci per c.c.m.

—	Staphylo-phagocytic index.	—	Staphylo-phagocytic index.
Unvaccinated blood	1	Bloods vaccinated with staph.	
Bloods vaccinated with staph.—		per c.c.m.—	
Circ. 2,500 per c.c.m.	1·3	Circ. 10,000 ..	1·9
,, 5,000 ..	1·5	,, 40,000 ..	2·1
		,, 80,000 ..	1·55

IV. Portions of the same blood were inoculated by the same technique with typhoid vaccine, the phagocytic power being afterwards tested with staphylococcus.

—	Staphylo-phagocytic index.	—	Staphylo-phagocytic index.
Unvaccinated blood	1	Bloods vaccinated with typhoid bacilli per c.c.—	
Bloods vaccinated with typhoid bacilli—			
Circ. 5,000 per c.c.m.	1·4	Circ. 20,000 ..	0·6
,, 10,000 ..	1·45	,, 40,000 ..	0·8

V. Same blood, same technique, and same typhoid vaccine were employed.

Staphylo-phagocytic index.

Unvaccinated blood	1	1
(Circ. 1,500 per c.c.m. typhoid bacilli ..	1·65	
Blood vac- ..	3,000	1·7
cinated with ..	6,000 ..	1·0
,, 12,000	0·85

VI. Same blood, the same technique, and the same typhoid vaccine were employed.

—	Staphylo-phagocytic index.	—	Staphylo-opsonic index.
Unvaccinated blood ..	1	Serum of unvaccinated blood ..	1
Blood vaccinated with typhoid bacilli—		Serum of blood vaccinated with typhoid bacilli—	
Circ. 4,000 per c.c.m.	1·25	Circ. 4,000 per c.c.	1·95
,, 8,000 ..	1·1	,, 8,000 ..	1·75

VII. Here a series of volumes of L. C.'s defibrinated blood were implanted with typhoid vaccine and were then immediately centrifugalised. The serum was then quickly pipetted off, and was tested for opsonic power by adding washed corpuscles and staphylococcus suspension.

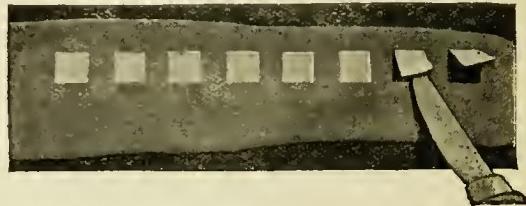
—	Staphylo-opsonic index.	—	Staphylo-opsonic index.
Serum from unvaccinated blood ..	1	Serum from blood vaccinated with typhoid bacilli—	
Serum from blood vaccinated with typhoid bacilli—		12,500 per c.c.m.	1·19
3,000 per c.c.m. ..	1·2	25,000 ..	1·2
6,250	1·38	50,000 ..	1·0

It will be seen that here in vitro, as in experiment I. *in vivo*, opsonic substances are delivered into the blood immediately—that is to say, they are furnished within the time that it takes to separate the serum from the corpuscles.

DERIVATION OF BACTERICIDAL AND OPSONIC SUBSTANCES FROM THE LEUCOCYTES.

The leucocytes provide the bactericidal and the opsonic substances that appear in the serum when epiphylactic response is evoked in the blood. To make sure of this we must first isolate the leucocytes from the other elements of the blood, must then make graduated additions of vaccines to serum (or other

FIG. 4.—A.



Method of making paraffin-framed emulsion cells.

B.



Blood imposed on emulsion cells.

menstruum), bring this into contact with the leucocytes, and then test its opsonic and bactericidal power. These requirements—and, of course, only the first presents any technical problem—are, as a matter of fact, easily satisfied. The leucocytes can be obtained entirely separate, for they will, if provided with facilities for doing so, emigrate from the blood. All they require is congenial warmth, a meshwork of fibrin to travel along, and a firm surface—such, for example, as the glass floor of an "emigration cell" or the walls of a capillary pipette—to collect upon.

Where emigration cells, such as are shown in Fig. 4 A are employed, these are, as in Fig. 4 B, filled brim full with blood taken direct from the finger.² After incubation in a moist chamber for one-half to three-quarters of an hour the clots are washed off with physiological salt solution. Where capillary pipettes are employed, the blood from the finger is drawn up into the stems, these are sealed up at the point, the barrel is then half filled with physiological salt solution, and then the blood is incubated for from half to three-quarters of an hour. After this a

² Wright and Colbrook: Technique of the Teat and Capillary Glass Tuho, Constable, 1921.

collapsed teat is fitted to the pipette, the tip of the stem is broken across, and the clot is slowly drawn up into the barrel and is then evacuated through the butt end along with the salt solution.

An emigration cell whose floor is coated with emigrated leucocytes may conveniently be called a *carpeted cell*, and a capillary tube similarly coated may be called a *lined tube*—the terms *uncarpeted* and *unlined* would then serve to denote control cells and capillary tubes with blank floors and walls.

The following experiments were carried out:—

I. Carpeted and uncarpeted emigration cells. A. E. W.'s leucocytes and serum. Here a 70-, a 300-, and a 600-fold dilution were made from a broth culture of staphylococcus. Of the first 5 c.mm. were implanted into 100 c.mm. of serum, and of the second and third 2.5 c.mm. into 50 c.mm. In each case half of the implanted serum was imposed on a carpeted and half on an uncarpeted cell. The two portions of serum were then, after a fixed interval, during which they were kept in a moist chamber on the bench, aspirated into fine capillary pipettes. These pipettes were then incubated for 24 hours, and the colonies which developed were counted.

(1) Serum with the heaviest implantation (which works out as 2200 staphylococci per c.em.).

	Colonies.
25 c.mm. { placed on an uncarpeted cell and imme- } 52	diately re-aspirated furnished } 56
25 c.mm. { placed on a carpeted cell and re-aspirated } 26	after 45 mins. furnished } 15

(2) Serum with the intermediate implantation (which works out as 520 staphylococci per c.em.).

	Colonies.
25 c.mm. { placed on an uncarpeted cell and re-aspirated } 13	after 45 mins. furnished }
25 c.mm. { placed on a carpeted cell and re-aspirated } 7	after 45 mins. furnished }

(3) Serum with the lightest implantation (which works out as 160 staphylococci per c.em.).

	Colonies.
25 c.mm. { placed on an uncarpeted cell and re-aspirated } 4	after 45 mins. furnished }
25 c.mm. { placed on a carpeted cell and re-aspirated } 2	after 45 mins. furnished }

(4) A supplementary volume of serum with the same implantation as in (2).

	Colonies.
25 c.mm. { imposed on an uncarpeted cell and re-aspirated } 14	after 45 mins. furnished }
25 c.mm. { imposed on the carpeted cell employed in } 2	(1) and re-aspirated after 45 mins. furnished }

II. Carpeted and uncarpeted emigration cells. I. F.'s leucocytes and serum. Same technique.

Serum implanted with circ. 4800 staphylococci per c.em. imposed on an *uncarpeted* cell and aspirated after 5 mins. furnished innumerable colonies.

Serum implanted with circ. 4800 staphylococci per c.em. imposed on a *carpeted* cell and aspirated after 5 mins. furnished 79 colonies.

Serum implanted with circ. 1200 staphylococci per c.em. imposed on an *uncarpeted* cell for 5 mins. furnished 30 colonies.

Serum implanted with circ. 1200 staphylococci per c.em. imposed on a *carpeted* cell for 5 mins. furnished 14 colonies.

Serum implanted with circ. 520 staphylococci per c.em. imposed on an *uncarpeted* cell for 5 mins. furnished 13 colonies.

Serum implanted with circ. 520 staphylococci per c.em. imposed on a *carpeted* cell for 5 mins. furnished 2 colonies.

The possibility that the diminution in the number of microbes in the serum which had been imposed upon carpeted cells might be due to a percentage of the microbes sticking to the leucocytes is negatived by considering the effect of implanting in duplicate into broth progressive dilutions of a staphylococcus culture, and explanting the one series of volumes directly, and the other after imposition upon carpeted cells. An experiment of this kind gave the following result:—

Series of volumes of broth explanted directly on to agar gave 33, 14, 7, 5, and 4 colonies—in all 63 colonies.

A similar series explanted on agar after imposition on a carpeted cell gave 33, 20, 10, 2, and 1 colony—in all 66 colonies.

III. Carpeted and uncarpeted emigration cells, and also lined and unlined capillary pipettes. A. E. W.'s leucocytes and 30 c.mm. volumes of serum. A 120-, a 240-, a 480-, and a 960-fold dilution were made of a broth culture of staphylococcus. 2.5 c.mm. volumes of the last two dilutions were implanted into 50 c.mm. volumes of broth which were then aspirated into very fine capillary tubes. The two heavier implanted volumes furnished 55 and 54 colonies; (a similarly implanted volume of serum furnished 47). The two more lightly implanted volumes of broth furnished 25 and 27 colonies.

A series of 30 c.mm. volumes of serum were now implanted with 2.5 c.mm. of the different staphylococcus suspensions; were then imposed in each case for 30 minutes on

carpeted emigration cells, and then drawn up into capillary tubes and incubated.

Vol. 1 implanted from the 120-fold dilution of staph. (i.e., with about 220 staph.) furnished ..	106
Vol. 2 implanted from the 240-fold dilution of staph. (i.e., with about 110 staph.) furnished ..	25
Vol. 3 implanted from the 480-fold dilution of staph. (i.e., with about 55 staph.) furnished ..	29
Vol. 4 implanted from the 480-fold dilution of staph. (i.e., with about 55 staph.) furnished ..	27
Vol. 5 implanted from the 960-fold dilution of staph. (i.e., with about 28 staph.) furnished ..	22
Vol. 6 implanted from the 960-fold dilution of staph. (i.e., with about 28 staph.) furnished ..	16

Calculation shows that here with an implantation of about 7500 staphylococci per c.em. we have a killing of 50 per cent., with an implant of about 3600 a killing of 77 per cent., with an implant of 1800 a killing of 50 per cent., and with an implant of 900 a killing of only 27 per cent.

Supplementary Experiment.—Three 50 c.mm. volumes of serum, implanted respectively with 2.5 c.mm. of the 120-, 240-, and 480-fold dilution of the staphylococcus culture, were drawn up into lined capillary tubes. All these volumes remained sterile. This, if we take the figures for the heaviest implantation, works out as equivalent to the killing of 7200 staphylococci per c.em. by the leucocytes obtained from 1 c.em. of blood.

IV. Lined and unlined capillary pipettes. A. E. W.'s leucocytes and 40 c.mm. volumes of his serum. A 150-, a 300-, a 600-, a 1200, a 2400-, and a 4800-fold dilution were made of a staphylococcus broth culture.

2.5 c.mm. of the 4800-fold dilution gave in serum in *unlined* tubes 17 and 18 colonies.

2.5 c.mm. of the 2400-fold dilution gave in serum in *lined* tubes 15 and 17 colonies. In *unlined* tubes in serum 27 and 31 colonies and in an *unlined* tube in broth 31 colonies.

2.5 c.mm. of the 1200-fold dilution gave in serum in *lined* tubes 19 and 15 colonies, as compared with a calculated number of 60 for serum in an *unlined* tube.

2.5 c.mm. of the 600-fold dilution gave in serum in a *lined* tube 21 colonies, as compared with a calculated number of 120 for serum in an *unlined* tube.

2.5 c.mm. of the 300-fold dilution gave in serum in a *lined* tube 15 colonies, as compared with a calculated number of 240 for serum in an *unlined* tube.

2.5 c.mm. of a 150-fold dilution gave in serum in a *lined* tube 10 colonies, as compared with a calculated number of 480 for serum in an *unlined* tube.

In the ease of the lined tubes the colonies that grew were in each case confined to the very narrow distal end of the capillary stem where the lining of leucocytes was probably deficient. Taking the figures for the most heavily implanted serum as they stand they testify to a killing of 63 per cent. with an implantation of 750 staphylococci per c.em., a killing of 72 per cent. with an implant of 1500 per c.em., a killing of 82.5 per cent. with an implant of 3000 per c.em., a killing of 94 per cent. with an implant of 6000 per c.em., and a killing of 98 per cent. with an implant of 12,000 per c.em.

V. Lined and unlined capillary pipettes. A. E. W.'s leucocytes and 50 c.mm. volumes of his serum. A 30-, a 150-, a 300-, a 600-, and a 2400-fold dilution were made of a broth culture of staphylococcus.

2.5 c.mm. of the 2400-fold dilution gave in serum in a *lined* tube 0 colonies, in *unlined* tubes 6 and 10 colonies.

2.5 c.mm. of the 600-fold dilution gave in serum in a *lined* tube 6 colonies, as compared with 27 in an *unlined* tube.

2.5 c.mm. of the 300-fold dilution gave in serum in a *lined* tube 7 colonies, as compared with a calculated number of 54 for serum in an *unlined* tube.

2.5 c.mm. of the 150-fold dilution gave in serum in a *lined* tube 0 colonies, as against a calculated number of 108 for serum in an *unlined* tube.

2.5 c.mm. of the 30-fold dilution gave in serum in a *lined* tube 100 colonies, as compared with a calculated number of 500 for serum in an *unlined* tube.

Here again the colonies (except in the heaviest implanted serum where they were distributed over the whole length) were all confined to the distal extremity of the pipette.

Leaving this out of consideration and taking the figures as they stand calculation shows that with an implantation of 10,000 staphylococci per c.em., 80 per cent. were killed; with an implant of 2000, 100 per cent.; with an implant of 1000, 87 per cent.; and with an implant of 500, 78 per cent.

We thus see that the laws governing epiphylactic response are the same for the leucocytes and serum as for the whole blood. Instead of obtaining with increasing implantations progressively less efficient killing (as we inevitably should if the bactericidal power were not enhanced by epiphylactic response) we obtain with increasing implantations (up to a certain limit) more and more effective killing.

I have now come to the end of what I have to say about the modus operandi of vaccines.

PART III.

RÉSUMÉ OF THE CONCLUSIONS REACHED IN THE FOREGOING INQUIRY.

WE have seen that epiphylactic response can be evoked in the blood by vaccines; that it can be evoked both *in vitro* and *in vivo*; that it is characterised by a sudden increase in the bactericidal and opsonic power of the serum, and that this increase is the result of a sudden evacuation of polytropic bactericidins and opsonins from the leucocyte. We have learned also something about the conditions under which epiphylactic response can be obtained. We have seen that when we vaccinate a normal blood *in vitro* with dead microbes and then test it with living, and also when we test a patient's blood which has been acted upon by antigens *in vivo*, the event will depend upon the total of antigen which has been brought into operation in the vaccinating procedure or auto-inoculation on the one hand, and the assaying procedure on the other.

With respect to the causative nexus between the application of excessive doses of vaccines and the development of apophylactic effects there is still a very serious lacuna in our knowledge. Seemingly the apophylactic effects produced by staphylococcus vaccine are different in origin and kind from those produced by typhoid and cholera vaccine. Whereas typhoid and cholera exert their apophylactic effect when brought into contact with serum, staphylococcus vaccine operates apophylactically only when brought in contact with the whole blood. This would seem to warrant the inference that when staphylococcus vaccine reduces the anti-bacterial power of the blood it does so not by adsorbing anti-bacterial elements from the serum, but by inciting the leucocytes to excrete into the serum some growth-favouring element.

Let me—asking you to remember that I cannot do any justice to the subject in the space now left to me—briefly indicate to you to what uses it may be possible to put the new information now in our possession.

PRACTICAL APPLICATIONS.

Preparation and Control of the Efficacy and Dosage of Vaccines.

We can put it to account in the first place in the preparation of bacterial vaccines. It will be clear that every point in connexion with the preparation of vaccines can be practically tested by the procedures that have been described above. For example, it will be possible to set on one side all those statistical inquiries which everyone proposes but nobody really carries out, and to ascertain directly whether one particular strain of microbes gives a better vaccine than another; whether one particular sterilising temperature is better than another; and whether it will be justifiable or unjustifiable to employ this or that particular vaccine in combating this or that infection. Further, we have here methods which will enable a comparison to be made between different kinds of vaccines. It will therefore henceforth be possible to call upon those who claim superiority for a particular brand of vaccine to show scientific justification for their claims. Lastly, it will be possible in the case of every vaccine to determine what is the dose which will give optimum response.

Here, however, we are launched upon a question which teems with difficulties and ambiguities. I will just indicate what they are and pass on. Consideration will show that when we speak of the optimum dose of a vaccine we really have in view a particular mode of administration and some approxi-

mate body weight (generally that of an average adult), and also a particular condition of the organism—the condition of being uninfected, or, as the case may be, lightly or heavily infected. Ordinarily, in speaking of the optimum dose, we have in view—and this is the particular point I wish here to consider—subcutaneous inoculation, this channel of administration being, upon the assumption that protective substances are elaborated by the tissues at the site of inoculation. Doubts, however, are cast upon the doctrine of the derivation of the protective substances from the tissues by the following general considerations: (1) When we implant lint impregnated with vaccines into the tissues, and then squeeze out that lint we obtain from it a lymph which has no whit more anti-bacterial potency than that obtained from a companion piece of plain sterile lint implanted elsewhere in the body. (2) The companion pieces of lint here in question are permeated with leucocytes, and the fluids obtained from them are indistinguishable, and contain in each case bactericidal substances which are in their range of action precisely similar to those which have been obtained directly from leucocytes. (3) Bactericidal and opsonic substances can, as has been seen above, be obtained by the operation of vaccines upon the blood *in vitro*—in other words, in conditions where the intervention of the tissues is definitely excluded.

When these facts are regarded hypodermic inoculation stands out under a very different light. Its paramount advantage would appear to lie in the circumstance that the vaccine is under favourable circumstances fed slowly and continuously into the blood. But where a large dose of vaccine is administered and carried into the blood too rapidly a formidable negative phase is bound to supervene. (It will be remembered that when the patient was not kept quiet after typhoid inoculation very severe negative phases used often to follow.) And, again, it will be clear that where a small dose of vaccine is given and convection into the blood takes place very slowly, the quantum of antigen in the blood may never arrive at the concentration required for the production of epiphylactic response. These uncertainties—which would, of course, be exaggerated if the method of oral administration were followed—are avoided by the intravenous administration of a dose of vaccine standardised so as to give the optimum number of microbes per c.c.m. of blood. With this system of dosage—which has here been followed throughout—there remain only the minor difficulties of estimating the patient's blood volume, and allowing for a progressive dissolution of microbes, and a consequent delivery of more than the anticipated quantum of antigen into the blood. But these minor difficulties need not be too minutely insisted upon.

EXTENSION OF THE RANGE OF THERAPEUTIC INOCULATION.

We saw at the outset that ordinary vaccine-therapy had proved ineffective in chronic streptococcal septicæmias, in many of the acute septicæmic conditions of local origin, and in phthisis when associated with hectic temperatures. And it might have been added that vaccine-therapy has in pneumonia, in typhoid, and in other similar infections given either uncertain or negative results. For example, the inoculation of pneumococcus in native pneumonia

¹ Wright: Lessons of the War, *THE LANCASTER*, March 29th, 1919, p. 496. The detailed experiments will be published hereafter.

patients in the hospitals of the Johannesburg mines² gave in our hands completely negative results. To the unsolved problems of the treatment of these infections we must now return, asking ourselves whether we are still compelled to make choice between a policy of non-intervention—disguised under the name of the expectant treatment—and a policy of purely empirical and ignorant intervention. It will be clear that we are no longer upon the horns of that dilemma.

To begin with—and this in itself is a step forward—we can now by a "vaccine-response test" undertaken upon the blood *in vitro* decide whether a patient is or is not capable of making immunising response. Where he is competent to make such response we can—and this is a further advance—ascertain to what dose of vaccine, intravenously inoculated, he will be able to make optimum response. Lastly, in the alternative of his not being able to make immunising response to a vaccine, we can straight off—for such cases do not admit delay—evoke epiphytactic response in a healthy human blood and resort to what I have called *immuno-transfusion*.

In addition to these therapeutic suggestions, which apply, as will be seen, to cases where life and death may already hang in the balance, two other proposals of perhaps lesser import may be brought up for consideration. The first relates to inhibitory inoculation in the incubation period of all zymotic fevers of bacterial or non-bacterial origin; the second to the possibility of arresting even after they have declared themselves, those minor but incapacitating infections grouped together under the heading of common colds. When we regard the fact that a non-specific epiphytactic response can be evoked instantaneously by the intravenous inoculation of appropriate doses of vaccine, it is not too venturesome to suggest that these desirable objects might possibly be achieved.

Only a small part of the domain which has here been rapidly reconnoitred has yet been explored, so it will perhaps be better to defer for a further communication any detailed account of successes achieved and disappointments experienced. But it will be indispensable to explain the method of going to work upon cases of septicaemia and acute infection, and to show how the methods of blood examination which have been described above are to be used in connexion with these cases.

PROCEDURE TO BE FOLLOWED IN DEALING WITH CASES OF SEPTICÆMIA AND ACUTE INFECTION.

For the scientific treatment of any grave infection there is required before all an equipment of principles. But that does not suffice. One requires in addition specific information with regard to the patient's condition. We require to know how he stands in the matter of the anti-bacterial power of his blood, and in the matter of its capacity for making immunising response to vaccines. And it is also essential to ascertain by direct means, with regard to each therapeutic intervention in its turn, whether it has been useful or ineffectual or hurtful. The laboratory methods described above can furnish us with this information. The first point to consider is how the anti-bacterial power of the patient's blood is to be gauged. Here the slide-cell inculturing procedure, and another technique which I shall have to describe to you, furnish the required data.

SLIDE-CELL INCULTURING PROCEDURE FOR MEASUREMENT OF HÆMO-BACTERICIDAL POWER.

The following examples give an idea of what can be learned from the slide-cell procedure. It may be explained that in each case graduated dilutions of a broth culture of staphylococci were implanted into 50 or 100 c.c.m. volumes of blood (vide supra, Figs. 2 and 3), and that the number of microbes implanted was enumerated by introducing similar quanta of

suspension into serum and enumerating the colonies which developed in this after incubation in capillary tubes or slide-cells.

OBSERVATION 1.—Patient suffering from Puerperal Streptococcal Septicaemia.

Number of staphylococcal colonies which developed in—	Number of staphylococci implanted respectively in volume—							
	1	2	3	4	5	6	7	8
1000	500	250	125	62	31	16	8	
Patient's blood ..	182	88	43	27	BD	BD	BD	BD

BD = Diffuse blue discolouration indicative of scattered bacterial growth.

OBSERVATION 2.—Patient suffering from Coryza.

Number of staphylococcal colonies which developed in—	Number of staphylococci implanted respectively in volume—							
	1	2	3	4	5	6	7	8
1120	560	280	140	70	35	17	8	
Patient's blood ..	48	31	31	4	4	1	0	0

OBSERVATION 3.—Patient suffering from Puerperal Septicaemia (M.M.).

Number of staphylococcal colonies which developed in—	Number of staphylococci implanted respectively in volume—			
	1	2	3	4
690		200	67	22
Patient's blood ..	51	22	7	4

OBSERVATION 4.—Patient suffering from Streptococcal Endocarditis.

Number of staphylococcal colonies which developed in—	Number of staphylococci implanted in volume—							
	1	2	3	4	5	6	7	8
320	160	80	40	20	10	5	3	
Patient's blood ..	30	7	3	2	2	2	2	2

OBSERVATION 5.—Patient suffering from Puerperal Streptococcal Septicaemia.

Number of staphylococcal colonies which developed in—	Number of staphylococci implanted in volume—							
	1	2	3	4	5	6	7	8
2000	1000	496	248	124	52	26	13	
Patient's blood ..	55	33	11	9	5	4	3	0

CHIASTIC PROCEDURE FOR MEASUREMENT OF PHAGOCYTIC POWER OF BLOOD, OPSONIC POWER OF SERUM, AND PHAGOCYTIC EFFICIENCY OF LEUCOCYTES.

What the slide-cell method supplies is, as has already been appreciated, a measurement of the haemo-bactericidal power obtained with different additions of living microbes. It does not tell us anything about the different factors upon which haemo-bactericidal power depends; nor does it, where a blood has a diminished bactericidal power, disclose to us the nature and reason of the default. A good deal of what is here left untold is supplied by the *chiastic method*. The procedure consists in preparing defibrinated blood from the patient and a normal man; separating the serum from each of the bloods; preparing from each washed corpuscles; and then making by what has come to be called the opsonic technique a series of four phagocytic mixtures, constituted as follows:—

(1) One volume of the patient's serum, 1 volume of his washed corpuscles, and 1 volume of any suitable bacterial suspension.

² Wright: On Pharmako-therapy and Preventive Inoculation Applied to Pneumonia in the African Native (Constable, London, 1914), pp. 83-85.

(2) One volume of the normal serum, 1 volume of the washed normal corpuscles, and 1 volume of the same bacterial suspension.

(3) One volume of the patient's serum, 1 volume of the normal corpuscles, and 1 volume of the bacterial suspension; and

(4) One volume of the normal serum, 1 volume of the patient's corpuscles, and 1 volume of the bacterial suspension.

It is, of course, from the interchange and crossing of the corpuscles and sera in phagocytic mixtures 3 and 4 that the appellation *chiastic* is derived. The phagocytic mixtures having been incubated, spread out upon slides, and counted in the accepted manner, we now collate and marshal the results in the following fashion:—

(a) By dividing the phagocytic count (i.e., the average microbic intake) of preparation 1 by that of preparation 2 we obtain the *phagocytic index of the patient's blood*.

(b) By dividing the phagocytic count of preparation 3 by that of preparation 2 we obtain the *opsonic index of the patient's serum*.

(c) By dividing the phagocytic count of preparation 4 by that of preparation 2 we obtain the *phagocytic efficiency of the patient's leucocytes*.

A few examples will show clearly what we learn from this triad of phagocytic functions.

Example 1.—Surgical nurse with acute streptococcal infection originating from puncture in finger—temperature 105° F.

	P.C.
Patient's serum + patient's washed corpuscles + S.S.	0·57
Normal " + normal " " + " ..	3·9
Patient's " + " " " + " ..	2·6
Normal " + patient's " " + " ..	1·36

S.S. = Streptococcus suspension. (Here, and in all the subsequent cases, a streptococcus vaccine diluted suitably supplied the suspension employed.) P.C. = Phagocytic count.

Phagocytic index of patient's blood, 0·15; opsonic index of patient's serum, 0·65; phagocytic efficiency of leucocytes, 0·34.

Example 2.—Child, aged 3 years, with acute streptococcal infection and abscesses in joints and subcutaneous tissues.

	P.C.
Patient's serum + patient's washed corpuscles + S.S.	0·2
A. E. W.'s " + A. E. W.'s " " + " ..	1·3
Patient's " + " " " + " ..	1·75
A. E. W.'s " + patient's " " " + " ..	0·18

Phagocytic index of patient's blood, 0·16; opsonic index of patient's serum, 1·3; phagocytic efficiency of leucocytes, 0·14.

Example 3.—Woman, aged 55 years, with streptococcal endocarditis.

	P.C.
Patient's serum + patient's washed corpuscles + S.S.	0·82
A. E. W.'s " + A. E. W.'s " " + " ..	4·05
Patient's " + " " " + " ..	0·44
A. E. W.'s " + patient's " " " + " ..	0·12

Phagocytic index of patient's blood, 0·2; opsonic index of patient's serum, 0·1; leucocytic efficiency, 0·03.

Results such as these can be registered in all acute septic infections. The premonitory signs of a grave infection would seem to be a reduction of the leucocytic efficiency, coupled with a rise in the opsonic index, and, as a result of some counterbalancing of the two factors just mentioned, a moderate fall in the phagocytic index. That type of result comes quite regularly under observation when we add to the blood *in vitro* excessive quanta of vaccine. We have already seen examples, and shall see others when we deal with the vaccine-response test. In the later graver stages of an infection the opsonic power is also reduced, and then the whole triad of phagocytic functions is prejudicially affected until they all, as in the last example, stand very little above the zero point.

It may here be pointed out, with regard to the leucocytic efficiency, that it appears to be reduced with regard to all microbes indiscriminately. Further, it may be noted that the leucocytes can recover from this heavily-poisoned condition. They can, as will be seen in a case presently to be cited, recover *in vivo* when the blood fluids which bathe them are rendered more wholesome; and they can recover *in vitro* when transported into, and allowed to stand in, wholesome serum. What has been said above of the blood changes associated with acute sepsis may be completed by saying that the sero-bactericidal power is not even in grave infections sensibly reduced.

INVESTIGATION OF THE PATIENT'S POWER OF IMMUNISING RESPONSE.

When the anti-bacterial power of the blood has been investigated and been found wanting, the next thing to do is to find out whether the patient

has any capacity of making immunising response, and with what vaccine and what dose he can make his optimum immunising response. This can be done by introducing graduated quanta of vaccine into a series of volumes of the patient's blood, and seeing whether there is any improvement in the haemo-bactericidal or phagocytic power of the vaccinated bloods, or in the sero-bactericidal or opsonic power of their sera. The information being wanted almost immediately, and there being no time for culture, investigations of the bactericidal power are ruled out. We are therefore restricted to the measurement of the phagocytic power of the inoculated bloods and the opsonic power of their sera.

Let me show you in a series of examples what we can learn from phagocytic vaccine-response tests. I may with advantage consider, first, an example which comes from the bulletin of treatment of the child whose blood furnished a moment ago an illustration of the working of the chiastic procedure.

Vaccine-Response Tests.

Example 1.—Blood of a child suffering from a generalised streptococcus infection and blood of a normal man who was about to furnish blood for immuno-transfusion into the child. March 7th, 1922, vaccine-response test conducted with desibrinated bloods.

		Strepto-phagocytic indices.	
		Patient.	Proposed donor.
Blood unvaccinated	...	0·3	1·0
Blood	20 stc. per c.c.m.	0·3	0·8
vaccinated	40 "	0·4	0·9
with	80 "	0·3	2·3
	160 "	0·2	0·9

Stc. = streptococci.

Example 2.—Blood of a patient suffering from acute streptococcus infection with a mastoid suppuration and A. E. W.'s blood. Feb. 9th, 1922.

		Strepto-phagocytic indices.	
		Patient.	A. E. W.
Blood unvaccinated	...	0·27	1·0
Blood	30 stc. per c.c.m.	0·1	1·7
vaccinated	60 "	0·09	1·5
with	90 "	0·03	1·45
	600 "	0·15	0·9

It is clear that when a patient's blood gives such results as these intravenous inoculations of vaccine are bound to do harm instead of good. These are cases for immuno-transfusion. The next example relates to the surgical nurse and to the self-same specimen of desibrinated blood which was employed for the first of the three chiastic tests cited above.

Example 3.—Patient with acute streptococcus infection spreading from a punctured wound and a temperature of 105° F.

The results recorded in the first column were obtained with phagocytic mixtures in which the sera and corpuscles of the unvaccinated and vaccinated bloods respectively were mixed with the streptococcus suspension; the results in the second column were obtained with phagocytic mixtures in which the sera of the unvaccinated and vaccinated bloods respectively were employed in combination with the streptococcus suspension and the corpuscles of normal blood.

		Strepto-phagocytic counts.	
		Blood.	Serum.
Blood unvaccinated	...	1·81	1·5
Blood	20 stc. per c.c.m.	1·9	2·0
vaccinated	40 "	1·6	4·7
with	80 "	1·4	1·7
	160 "	3·2	2·7

These results show (and the contrary might have perhaps been expected from the event of the chiastic test)³ that the patient's blood still possessed a con-

* Vide supra.

siderable capacity for immunising response, and that vaccination with 160 streptococci per c.cm. gave the best response. The patient was accordingly immediately inoculated intravenously with this dose of vaccine; in other words, with 700,000 streptococci.

Example 1.—Patient with chronic streptococcal septicaemia and endocarditis.

	Staphylo-opsonic index.
Blood unvaccinated ..	1·0
Blood vaccinated with 20 strep. per c.cm. ..	1·12
" " 50 ..	1·18
" " 100 ..	1·6
" " 200 ..	1·4
" " 1000 ..	2·35
" " 4000 ..	1·26

This patient was obviously a suitable subject for intravenous inoculation with streptococcus vaccine.

VERIFICATION BY LABORATORY METHODS OF IMPROVEMENT OBTAINED BY TREATMENT IN GRAVE BACTERIAL INFECTIONS.

In grave bacterial infection only three kinds of therapeutic intervention appear to hold out any promise. (1) First among these would come—but the procedures in question apply only where there is localised infection—the evacuation of collections of infected pus, the effective draining of the tissues which encase a putrid wound, and the ablation of foci of infection. Such procedures, of course, abolish that continuous and immoderate auto-inoculation which poisons the leucocytes and interferes with immunising response. Where mechanical procedures for the reduction of the volume of infection are inapplicable our choice would appear to lie between (2) inoculations—by preference intravenous inoculations—of vaccine, and (3) immuno-transfusion. But our scientific task is not completed when we have applied the particular form of treatment selected. A priori inference is in medicine an untrustworthy guide. And when verification is in question, the guarantee furnished by quantitative laboratory methods—though this cannot compete with the psychological impression produced upon eye-witnesses by sudden and dramatic clinical improvement—is that which finally counts. In view of this I have set out below examples which show in connexion with each of the three aforementioned kinds of therapeutic intervention evidence of the benefit obtained from it. The examples have here been chosen to furnish illustration of points not previously brought out, and in particular to exhibit the increased anti-bacterial power obtained.

Example 1.—Showing the improved phagocytic and opsonic power and increased vaccine response and leucocytic efficiency obtained by evacuating an abscess.

Child with generalised streptococcus infection and an unopened abscess in the elbow joint who had been treated by two successive immuno-transfusions, each with 25 c.cm. of blood, on March 21st and 24th, 1922.

March 27th—*Chiastic Test:* Phagocytic index of blood, 0·07; opsonic index of serum, 0·8; phagocytic efficiency of leucocytes, 0·1. Abscess was now opened.

March 28th—*Chiastic Test:* Phagocytic index of blood, 1·02; opsonic index of serum, 1·4; phagocytic efficiency of leucocytes, 0·9.

Vaccine-Response Test.

Strepto-phagocytic index.

Unvaccinated blood	1·0
Blood vaccinated with 20 strep. per c.cm.	1·3
" " 40	1·6
" " 80	1·0

To appreciate the improvement, the data of this vaccine response test should be compared with those registered with this child's blood before treatment was begun (vide Example 1 foregoing section). The child's night temperature, which had for three weeks before the first immuno-transfusion ranged up to between 103° and 105°, and which had after the first immuno-transfusion come down to below 102°, and after the second to under 101° F., came down after the opening of the abscess to between 99° and 100° and soon after became normal.

Example 2.—Showing increased opsonic power obtained by immuno-transfusion.

Patient with puerperal septicæmia. March 6th, 1922, immediately before immuno-transfusion, strepto-opsonic index 1·2, immediately after 2.

Example 3.—Showing increased phagocytic power obtained by immuno-transfusion.

Patient with grave streptococcal infection and mastoid suppuration. Feb. 7th, immediately before immuno-transfusion, strepto-phagocytic index 0·3, immediately after 1·2. In this latter case the patient, who had three hours before the immuno-transfusion had a rigor with a temperature of 106° F., and was lying cyanotic and semi-comatose, revived at once after the immuno-transfusion, the colour improving, the mental condition becoming normal, the patient remarking that she felt wonderfully better, eating ravenously, and sleeping quietly at night without requiring any administration of oxygen.

The same patient, after two further high evening temperatures. Feb. 9th, 1922, 24 hours before the second immuno-transfusion, strepto-phagocytic index 0·27; on the 10th, immediately before the immuno-transfusion, 0·9. Transfusion of blood vaccinated *in vitro* with 100 streptococci per c.cm. On the 11th, one day after, 0·7; on the 12th, two days after, 1·1. On the 14th, four days after, 1·3; on the 15th, five days after, 1·1.

Vaccine-Response Test. (This result is to be compared with that recorded in Example 2 of the previous section.)—On the 12th, unvaccinated blood per c.cm., strepto-phagocytic index 1·1; blood vaccinated with 20 streptococci per c.cm., 1·0; with 40, 1·3; with 80, 1·0.

In connexion with this immuno-transfusion the clinical report says: “The result of the transfusion was again magnificent, the oxygen inhalation being discontinued, while the patient's colour improved, and she took nourishment well and passed a very good night.” After very slight rises of temperature treated by an inoculation of streptococcus vaccine the patient made a rapid recovery.

Example 4.—Showing the increased haemo-bactericidal power obtained in the case of donor by the intravenous inoculation of 160,000 streptococci, and in the recipient by the immuno-transfusion of 500 c.cm. of the donor's blood drawn off half an hour after he had been inoculated intravenously.

Patient suffering from necrosis of frontal bone and generalised infection originating in an operation on the nasal sinuses. The slide-cell inculting procedure was employed, graduated doses of staphylococci being implanted into 50 c.mm. volumes of blood.

Donor's blood.				Patient's blood.			
Before inoculation.		Half-hour after inoculation.		Before immuno-transfusion.		24 hours after immuno-transfusion.	
Imp.	Surv.	Imp.	Surv.	Imp.	Surv.	Imp.	Surv.
206	11	496	10	264	42	164	3
121	6	145	1	137	20	115	1
48	0	72	0	67	6	50	0
23	1	32	0	49	5	23	0
9	0	17	0	22	3	10	0
10	0	9	0	13	0	0	0
1	0	4	0	3	2	2	0
0	0	0	0	2	0	2	0

Imp. = Implanted.

Surv. = Survived.

Synopsis of Results.

1. Into the donor's blood taken before inoculation in all 418 staphylococci were implanted, and 4·3 per cent. developed into colonies.

2. Into the donor's blood taken half an hour after inoculation in all 775 staphylococci were implanted and 1·3 per cent. developed into colonies.

3. Into the patient's blood taken immediately before immuno-transfusion in all 557 staphylococci were implanted and 11 per cent. developed into colonies.

4. Into the patient's blood taken 24 hours after immuno-transfusion in all 366 staphylococci were implanted and 1·1 per cent. developed into colonies.

Comment.

In connexion with these and all other data furnished by the slide-cell inculting procedure, it is perhaps advisable to return to a matter which has already been discussed, and to make clear the exact import of the figures. It must be kept in view that the assaying procedure employed is not an assaying procedure pure and simple, but also incidentally a supplementary vaccinating procedure. It follows that the improvement shown in the case of the donor

is really the resultant of two vaccinations—a vaccination in vivo and a superadded vaccination in vitro—and in the same way the improvement shown in the case of the patient is the resultant of an immuno-transfusion in vivo and a superadded vaccination in vitro. That being so, we are not entitled to claim for the intravenous inoculation and the immuno-transfusion that it did all that the figures in the tables might at first sight suggest. But we shall have made the proper abatement from that claim if we say this: The bloods of the donor and patient respectively have been so improved by our operations that they would, if they afterwards encountered an infection equivalent to an implantation of in the one case 10,000 (496×20) or less staphylococci per c.c.m., and in the other case to 3200 (164×20) or less staphylococci per c.c.m., have shown an improvement equivalent to those set out in the tables in connexion with these figures.

Example 5.—Showing increased sero-bactericidal power obtained by immuno-transfusion of 500 c.c.m. of defibrinated blood obtained from a donor who had four hours before been inoculated intravenously with 150,000 streptococci.

Patient suffering from a chronic hectic temperature supervening upon a staphylococcal infection of both breasts which had led to their amputation.

		Number of staphylococcus colonies which developed in 50 c.c.m. volumes of serum.
Serum of	immediately before immuno-transfusion	36
blood drawn off	5 minutes after immuno-transfusion	28
	1 hour after immuno-transfusion	21

This is equivalent to an increased killing power of 240 staphylococci per c.c.m. Consideration will show that this method of assay excludes the possibility of any supplementary epiphylactic response in vitro.

Example 6.—Showing increased sero-bactericidal power to staphylococcus obtained by the immuno-transfusion of 500 c.c.m. of blood vaccinated in vitro with 30 streptococci per c.c.m.

Patient with a chronic pyrexia following on a series of local streptococcal infections.

		Number of staphylococcus colonies which developed in 20 c.c.m. volumes of serum.
Serum from blood drawn 21 hours before immuno-transfusion	{ 36 } 33½	
Serum from blood drawn immediately before	{ 41 } 40	
Serum from blood drawn 5 minutes after immuno-transfusion	{ 21 } 26	
Serum from blood drawn 21 hours after immuno-transfusion	{ 24 } 22	

Calculation shows that here the serum after immuno-transfusion killed 700 more and the serum 21 hours after 900 more staphylococci per c.c.m. than before.

Example 7.—Demonstration by the implanting and explanting method of increased hæmo-bactericidal power obtained by immuno-transfusion of 500 c.c.m. of defibrinated blood from a donor who had half an hour before been inoculated intravenously with 170,000 streptococci.

Patient with puerperal streptococcal septicæmia.

Number of staphylococci implanted per 5 c.c.m. of blood.	Number of colonies which developed on agar when 5 c.c.m. of blood drawn before immuno-transfusion was explanted.		Number of colonies which developed on agar when 5 c.c.m. of blood drawn immediately after immuno-transfusion was explanted.	
	Immediately.	After $\frac{1}{2}$ hour.	Immediately.	After $\frac{1}{2}$ hour.
55	32	94	38	48
29	10	53	15	11
10	6	16	10	7
7	5	6	8	1
4½	3	2	5	2
105	56	171	76	69

We have here the results of two separate assays. When assayed by implanting and explanting immediately (the comparison is here between columns 1, 2, and 4), the blood drawn off immediately after immuno-transfusion showed a decline rather than an improvement. When assayed by implanting and explanting after half an hour (the comparison is here between

columns 1, 3, and 5), the blood drawn off immediately after immuno-transfusion shows great improvement. While in the blood drawn off before immuno-transfusion 105 microbes had increased to 171, and in the blood drawn off after, the 105 had decreased to 69.

I may just advert to one further point of theoretical interest in connexion with immuno-transfusion. This is that when the donor, or his blood, has been inoculated with too large a quantum of vaccine, or where insufficient time has intervened between inoculation and transfusion, instead of a positive, a negative phase, such as that which would have been produced by a direct inoculation of vaccine, will supervene.

I may now pass from the illustration of the effects of immuno-transfusion to illustrate those of the direct inoculation of vaccines into patients whose blood has after examination by the chistic or vacucue-response test, or on general clinical grounds, been adjudged to be capable of making immunising response. It will—for we are here dealing with blood conditions which are, of course, fundamentally the same as those obtaining in health—suffice here to adduce a couple of examples.

Example 8.—Increased hæmo-bactericidal and sero-bactericidal power obtained by the intravenous inoculation of 700,000 dead streptococci (160 per c.c.m. of patient's blood).

Surgical nurse suffering from an acute streptococcal infection originating in a punctured wound of the finger. Temperature 105° F. Measurement of hæmo-bactericidal power by implanting staphylococcus by the wash and after-wash procedure into a sequence of 10 c.c.m. volumes of blood in a long capillary tube.

	Number of colonies which developed in volumes—					Total in—
	1	2	3	4	5	
10 P.M.: Blood immediately before the intravenous inoculation	+ 6	5	2	2	2	21·5
Blood 3 mins. after the inoculation	+ 16	8	4	0	0	54·0
	+ 6	0	0	0	0	7
	+ 6	2	0	0	0	17·5

The sign + indicates that the colonies were too numerous for accurate enumeration.

Sero-Bactericidal Power (same technique).

	Number of colonies which developed in volumes—					Total in—
	1	2	3	4	5	
3 P.M.: Serum from blood drawn 7 hours before intravenous inoculation	+ 20	12	5	1	1	37
	+ 17	14	3	1	1	100·0
	+ 19	12	5	2	2	
10 P.M.: Serum from blood drawn immediately after inoculation	+ 12	9	0	0	0	22·5
	+ 17	4	3	0	0	55·0
12.30 A.M.: Serum from blood drawn 2½ hours after inoculation	+ 10	5	2	0	0	14·5
	+ 9	2	1	0	0	36·0

The patient's temperature by next morning had come down from 105° to 99°, and she was quite out of danger. The finger afterwards began to suppurate and was incised, and the patient made a rapid recovery.

Example 9.—Increased hæmo-bactericidal power obtained by intravenous inoculation of 30,000 streptococci.

Patient suffering from streptococcal septicæmia and endocarditis. Slide-cell incultering method.

Number of staphylococci implanted.	Number of colonies which developed in blood drawn off—	
	Before intravenous inoculation.	20 mins. after intravenous inoculation.
160	31	12
80	16	9
40	7	7
20	3	3
10	4	0
5	0	0
315	61	31

Example 10.—Increased sero-bactericidal power obtained by intravenous inoculation of 125,000 dead staphylococci (50 per c.cm. of patient's blood).

Child with streptococcal endocarditis (M.F.). June 19th, 1922, sera from bloods drawn off before and at intervals after inoculation. Measurement of sero-bactericidal power by implantation of 2.5 c.mm. of staphylococcus suspension into 50 c.m.m. samples of serum.

	Number of samples tested.	Average number of colonies.
Serum before inoculation ..	6	67.5
," $\frac{1}{2}$ hour after inoculation ..	3	41.5
," 2 hours ..	3	49.5

Calculation shows that here the serum half an hour after inoculation killed per c.c.m. 520 more, and two hours after inoculation 360 more staphylococci than before.

Example 11.—Increased sero-bactericidal power obtained by intravenous inoculation of 130,000 dead streptococci (55 per c.cm. of patient's blood).

Same patient and the same procedure.

Two volumes of serum immediately before inoculation furnished 82 and 91 colonies, average 86.5.

Two volumes of serum half an hour after inoculation furnished 82 and 99 colonies, average 90.5.

Two volumes of serum two hours after inoculation furnished 78 and 88 colonies, average 83.

One volume of serum four hours after inoculation furnished 32 colonies.

Calculation shows that the serum four hours after inoculation killed 1090 more staphylococci per c.c.m. than before.

Example 12.—Increased hemo-bactericidal power to staphylococcus obtained by the inoculation of 200,000 typhoid bacilli 80 per c.cm. of patient's blood.

The same patient. Measurement of haemo-bactericidal power by implantation of 2.5 c.mm. of progressive dilutions of staphylococcus culture into 50 c.m.m. samples of blood and incubating these in slide-cells.

	Number of colonies of staphylococcus which developed in volumes—									Total.
	1	2	3	4	5	6	7	8	Total.	
Blood drawn immediately before inoculation ..	32	16	9	4	5	1	0	0	67	
Blood after inoculation ..	23	7	3	1	0	1	1	1	37	

The number of staphylococci implanted was the same for the two bloods.

Example 13.—Increased hemo-bactericidal power obtained by the intravenous inoculation of 40,000,000 staphylococci (8000 per c.cm. of patient's blood).

Patient with chronic hectic temperature following upon staphylococcal infection of the breasts. Procedure as in Example 12.

	Number of colonies of staphylococci which developed in volumes—									Total.
	1	2	3	4	5	6	7	8	Total.	
Blood drawn immediately before inoculation ..	31	13	7	3	2	0	1	1	58	
Blood one hour after inoculation ..	6	2	1	1	0	0	0	0	10	

The number of staphylococci implanted was the same for the two bloods.

CONCLUSION.

I have now finished with the setting out of the data of individual experiments, and would, in conclusion, set out two considerations of a more general order. The first has reference to the question of the therapeutic prospects here opened up. The experiments which have been set out have brought into prominence the fact that in immunisation quantitative considerations dominate the situation. When we want to evoke immunising response in the blood we must employ one particular range of doses. And when we want to ascertain what has been achieved we must again employ one particular range of doses. Once that principle has been accepted, and we realise that in each case only a definite measure of additional anti-bacterial power is engendered, and that with it only such and such an additional number of microbes can be killed, it becomes possible to get down to figures.

In the experiments incorporated in this paper the increased anti-bacterial power engendered runs, as calculation shows, generally into the killing of several additional hundreds or exceptionally several additional thousands of staphylococci per c.c.m. of blood or serum. It never gives us an additional killing of tens or hundreds of thousands. These figures, then, give us our upper and lower limits. Within that range there is, as reflection will show, much useful therapeutic work to be accomplished. But we must not close our eyes to the fact that, just as there is an assaying dose of living microbes which no immunised blood can contend against, so there must be a very definite limit to the volume of infection which intravenous inoculation of vaccines, or immuno-transfusion can cope with. And in computing that volume of infection we must take into reckoning not only the microbes actually circulating in the blood (those even in very severe cases of streptococcal septicæmia do not amount to more than a very few hundreds of microbes per c.c.m.), but the total bacterial population in the internal organs, which may run into indefinite millions.

The second point—which comes in appropriately at the end of this paper, which began by showing how always a new code of principles comes and supersedes or modifies its predecessor—is that it behoves us to realise that in every code of principles there are tenets based only upon inference as well as tenets based upon direct experiment. But while we regard that, we should also keep before us that in every succeeding code those elements which rest on inference grow less and less, and those which rest directly upon fact—those which are established by laboratory methods—grow more and more. So that in the end, in every science, enduring principles will be reached.

In conclusion, I would express to the Medical Research Council grateful acknowledgments for those external circumstances which allow of the undertaking of protracted and undisturbed researches, and for the valuable aid of Dr. Leonard Colebrook.



H.C.